PB-222 384

ELECTRICAL STIMULATION OF MICROBIAL WASTE TREATMENT

J. S. Schultz

Michigan University

Prepared for:

Environmental Protection Agency

June 1973

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W73.12503

EPA R2-73-278
June 1973
PB 222 384

ELECTRICAL STIMULATION OF MICROBIAL WASTE TREATMENT

by

J. S. Schultz
University of Michigan
Ann Arbor, Michigan

Grant #17050 DGJ

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OFFICE OF RESEARCH AND MONITORING
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

W73-12503

4. Title
ELECTRICAL STIMULATION OF MICROBIAL WASTE TREATMENT

5. Report Date
6.
8. Performing Organization Report No.
10. Project No.

7. Author(s)
Schultz, J. S.

9. Organization
Michigan University, Ann Arbor
Department of Chemical Engineering

11. Contract/Grant No.
EPA-17050 DGJ

12. Sponsoring Organization

13. Type of Report and Period Covered

15. Supplementary Notes
Environmental Protection Agency Report Report
EPA-R2-73-278, June 1973
P. 46, 18 fig., 3 tab.

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In batch fermentation experiments some stimulation of growth and sugar utilization was found over a current range of 40 to 120 ma DC, however, the amount of stimulation was within the range of experimental variations. In continuous fermentation, cell growth was not significantly stimulated by electric fields. Electrical fields caused some increase in the accumulation and transport of galactose by E. coli. (EPA)

17a. Descriptors
*Electricity, *Fermentation, *Waste water treatment, Electric Fields, Electric Currents, Bacteria, Yeast, Growth Rates, *Waste Treatment, Cultures, Microorganisms, *Metabolism

17b. Identifiers
*Stimulation, Membrane Transport, Batch Culture, Continuous Culture

17c. COWRR Field & Group 05D

Available from the National Technical Information Service as PB , $3.00 in paper copy, $0.95 in microfiche

19. Security Class. (Report)
20. Security Class. (Page)
21. No. of Pages
22. Price

Send To:
WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D. C. 20240

Abstractor
Institution

WRSIC 102 (REV. JUNE 1971)

GPO 913.201

ABSTRACT

This project has been directed towards the evaluation of the effect of electrical fields, AC and DC, on the metabolism and growth of microorganisms. Test systems used were batch culture, (aerobic and anaerobic), continuous culture, and the use of genetic mutants for membrane transport studies.

Voltages were applied to cell suspensions with inert platinum and carbon electrodes, and were limited to levels below the onset of electrolysis of water. Care was exercised in maintaining constant temperatures for comparative experiments, since one direct effect of applied currents was resistive heating of the solutions.

The growth medium was designed so that the carbon source (usually glucose) was the limiting nutrient. For most of the studies, analysis of cell growth and glucose utilization was used to evaluate the effects of electric fields.

In batch fermentation experiments some stimulation of growth and sugar utilization was found over a current range of 40 to 120 ma DC, however, the amount of stimulation was within the range of experimental variations. In continuous fermentation, cell growth was not significantly stimulated by electric fields.

Experiments to test the effect of fields on the galactose transport system of E. coli showed some increase in the accumulation and transport of this sugar.

Methods for automatic sampling and storage of samples for continuous cultures were developed in addition to procedures for obtaining kinetic model parameters from transient states in continuous cultures.

This report was submitted in fulfillment of Project Number 17050DGJ by the University of Michigan under the partial sponsorship of the Environmental Protection Agency.

Key Words: Electricity, electric field, electric current, fermentation, batch culture, continuous culture, bacteria, yeast, stimulation, membrane transport, growth

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SECTION I

CONCLUSIONS

Electric fields have a slight effect on the growth rate and substrate utilization rate of microorganisms in the logarithmic phase of batch growth.

Electric fields do not have a significant effect on metabolism and growth of yeast in continuous culture.

Electric fields have a slight stimulatory effect on the transport and accumulation of galactose into E. coli.

SECTION II

RECOMMENDATIONS

The studies reported here were on pure cultures only, and not designed to evaluate synergistic effects. The effect of electric fields on waste treatment processes should be tested directly in pilot plant operations to test the possibility of synergistic effect.

The effect of electric fields on specific cellular processes should be evaluated, as such experiments are more likely to lead to the mechanism of the effect than overall growth rate or glucose utilization.

Further theoretical and experimental work should be directed towards methods of obtaining kinetic data of continuous fermentations, operating in a transient mode.

SECTION III

INTRODUCTION

Over the last 30 years reports appearing in the scientific literature have convincingly demonstrated that electric currents affect the metabolism of biological cells. However, the conditions that are necessary and sufficient to stimulate cellular metabolism in microorganisms have not been clearly defined, nor has the mechanism of stimulation been elucidated.

Since electrical energy has been shown to accelerate the major aspects of microbial metabolism, i.e., growth, synthesis, and catabolism, it is apparent that the applications of this technique are as diverse as are the uses of the microorganism itself. The cell material provides food or food supplements, its catabolic activity is useful in processes such as waste treatment, and its synthetic capabilities are necessary for producing amino acids, vitamins, etc. This project is devoted to the investigation of electrical effects on microbial cells.

One of the prime considerations in water quality control systems is the treatment of wastes by biological methods. Microbial waste treatment methods offer advantages mainly because most organic matter is susceptible to attack by microbial cultures and because the waste material is converted to proteins and carbohydrates that can be reused. However, microbial treatment methods are relatively slow and require large retention volumes.

It may be possible to accelerate microbial metabolic rates by electrical stimulation at extremely low power levels, resulting in smaller packages to satisfy waste treatment requirements and making microbial waste treatment a more attractive proposition for current and future plant expansion. Electrical interaction with microbial systems may also lead to methods for controlling the rate of microbial metabolism; such rate control could be useful for accommodating varying demands on the waste treatment system.

The primary aims of this project are:

1. To find out whether metabolic stimulation is caused by the presence of electric fields or by the passage of Faradaic electric currents.

2. To evaluate the quantitative relationship between electrical stimulus and metabolic response.

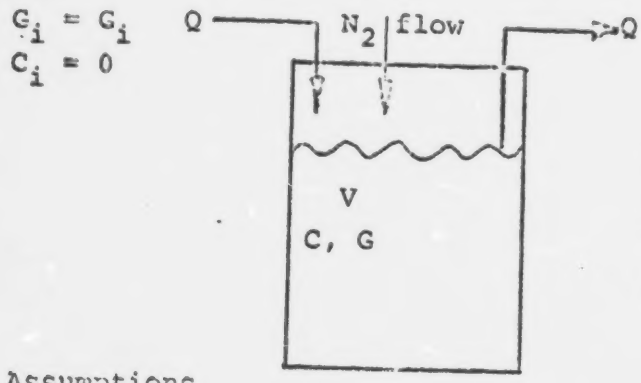
3. To explain the mechanism of electrical stimulation at the cellular level.

The basic experimental procedure was to grow microorganisms in batch and continuous (steady-state) culture and to subject the cells to electric currents and fields. The effects will be related to gross measures of microbial activity as cell density or substrate usage and where possible the kinetic parameters of a mathematical model for microbial growth and metabolism.

Either batch or continuous fermentation experiments may be used to substantiate or repudiate claims that electrical currents can stimulate microbial growth. Batch fermentation has the advantages of shorter experimental time, less danger of contamination and simpler sampling techniques. However, the data are an instantaneous comparison between control and experimental conditions. Data from the exponential growth region may be used to predict growth rate constants. However, there is no guarantee that batch data can be translated into accurate predictions of a continuous process such as the activated sludge waste treatment. Continuous fermentation experiments can more closely predict such processes. Continuous fermentation does require, however, longer experimental times, more difficult sterilization procedures and more sophisticated sampling techniques.

The length of the experiment is perhaps the most serious of the problems associated with continuous fermentation. Many experimentors are willing to concede that steady-state has been reached only after 24 hours or more of constant conditions. Early batch fermentations of yeast in a glucose media indicate that any potential stimulation of growth would be very slight if at all (most likely 15% or less). After steady-state has been reached, one would not expect large deviations of conditions during an application of electric current. Therefore, non steady-state equations can be used to account for the transient response toward a new steady-state position and eliminate much of the waiting.

The general mathematical models which apply to such non-steady-state conditions are as follows:



V is volume (constant)

Q is flow rate

C is cell concentration

G is glucose concentration (limiting substrate)

Assumptions

1. Defined media - glucose is the only source for growth carbon and its is present in limited concentrations.
2. The flow rate is constant during any specific run.
3. The fermentor is well mixed (the concentrations are uniform).

Mass Balance Relations

(cells) $$(G_i)(Q) - (C)(Q) + (R_c)(V) = (dC/dt)(V) \quad (1)$$

(glucose) $$(G_i)(Q) - (G)(Q) - (R_g)(V) = (dG/dt)(V) \quad (2)$$

where R_c is that rate of formation of cells and R_g is the rate of utilization of glucose

Rate Relations

R_c is proportional to C* where C* is the concentration of viable cells.

Each cell is able to reproduce between 30 and 100 times before viability is lost. In a continuous fermentation with this system and Saccharomyces cerevisiae there is less than 1% chance of finding cells which have reproduced more than five times during a random selection of a yeast cell. Assuming that any electrical experiments are relatively mild and cause no loss in viability, it is logical to expect that no significant error will be introduced by assuming that C* is identical to C.

Therefore:

$$R_c = (\mu)(C) \quad (3)$$

where μ is the growth rate constant

If we define the cell yield "Y" as the mass of cells produced per mass of glucose consumed, at any instant in time we have:

$$R_c/R_g = Y \tag{4}$$

If we set Q/V equal to D, the dilution rate, equation one may be re-written as:

$$R_c = dC/dt + (C)(D) \tag{5}$$

And Equation two may be re-written as:

$$R_g = (G_i - G)(D) - dG/dt \tag{6}$$

At steady-state dC/dt and dG/dt are zero and equations five and six may be substituted into equation four to give the familiar yield equation which is attributed to Monod:

$$C/(G_i - G) = Y \tag{7}$$

If we solve equation three for μ, we have

$$\mu = R_c/C \tag{8}$$

Equations four, five, six and eight provide the basis for the unsteady-state data analysis.

In order to correlate all the data, one needs not only mathematical models, but also microbial growth models. These growth models should relate μ to various experimental conditions such as cell concentration and limiting substrate (glucose) concentration.

Models for μ

Several models have been proposed for μ. Some of them are presented below.

$\mu = \mu_{max}$ Malthus

where μ_{max} is the maximum growth rate without limiting substrate

$\mu = \mu_{max}(1 - B*C)$ Verhulst & Pearl

where B is a constant

$\mu = \mu_{max}(G)$ M'Kendrick & Pai

$\mu = \mu_{max}(G)/(K_s + G)$ Monod

where K_s is a constant

$\mu = \mu_{max}(1 - e^{-G/K_s})$ Teissier

$\mu = \mu_{max}(1/1(1 + (K_s/G)^x))$ Moser

where x is a constant

$\mu = \mu_{max}(G/(K_s*C + G))$ Contois

Once a satisfactory growth model is chosen for control conditions, the various constants may be determined. During the application of electric current, the constants may be again determined and any stimulation reported accordingly.

When experiments on the effect of electric currents in batch culture showed minor changes in gross measures of cell growth and metabolism it was decided to study a more specific response of the cell. The rationale of choosing this approach is the possibility that cell metabolic control mechanisms may compensate for changes induced by electrical fields, and gross measures of metabolism may not be sensitive enough to show the effects clearly. The transport of galactose into a galactosekinase-less mutant of E. coli (NL 32,400) was chosen for study because it has been shown (Horecker, 1960) that for this particular mutant a specific permease is present for the transport of galactose. By measuring the effect of electric fields on transport of galactose, we have an indirect way of estimating the effect of electric fields on the specific proteins involved in this subsystem of the cell's metabolic machinery.

SECTION IV

LITERATURE REVIEW

One of the earliest reports on the effect of electrical currents was by Thiele and Wolf (1899). They subjected various species of bacteria to DC and AC currents (100 cps). The bacterial spores were dispersed in a gelatin plug, through which the electric current was passed by means of salt bridges. After the treatment, which was controlled at 10°C, the spores were plated out. The treated cultures grew out as well as the controls. No data were given as to rates of growth.

Palladin and Millyak (1914) reported that the rate of fermentation of sucrose as measured by CO_2 evolution increased when an AC current was passed through the solution. DC current was inhibitory.

Klieber (1925) investigated the effects of DC and AC currents on a yeast fermentation at different temperatures. Essentially no effect on CO_2 production was found. AC slightly stimulated and DC slightly inhibited alcohol production as measured by changes in specific gravity.

Schieblich and Schulze (1926) studied the growth of several strains of bacteria under the influence of AC current at a controlled temperature. In most cases no significant effects were noticed, but in one case there was a stimulation in growth as measured by dry weight.

H. Fritz (1929) found an inhibitory effect of high AC currents on yeast growth with both silver and platinum electrodes. He suggested that the inhibition was a result of the electrolytic formation of AG + Cl_2. The effect could be lessened with higher concentrations of yeast.

Hattingberg (1934) investigated the effects of current pulses on the respiration of red blood cells from various species of warm- and cold-blooded animals. The experiments were carried out in Warburg apparatus, and both oxygen uptake and CO_2 liberation were measured. Pulses of current from 2 to 8 ma were applied for 20 sec each minute for a half hour before and after a control period. Typically, oxygen uptake was increased when current was applied. The observed effect could not have been due to electrolysis of water, because a net decrease in gas volume was found instead of the increase in gas one would expect with electrolysis. In one experiment a red blood cell suspension was stimulated by current at various

times over a 19 hour period. In each instance an increase in O_2 uptake from 70 to 120% above a control was found. The absolute O_2 uptake rate decreased with time, but the effect of current increased with time.

The stimulatory effect of current on O_2 uptake was found with red blood cell samples from 5 different species. The stimulation ranged from 40% (pigeon rbc) to 140% (frog rbc).

The increase in oxygen uptake rate was also found to be more than proportional to the current applied, at 2 ma the rate increased 25%, at 8.5 ma it increases about 300%. In order to be sure that the increase in oxygen uptake was truly a result of enhanced metabolism and not due to physical factors such as greater binding of oxygen by hemoglobin, Hattingberg measured the respiratory quotient during both the control and stimulated periods. He found no change in respiratory quotient, a mole of CO_2 being formed for each mole of O_2 used. As a further check to see if only respiratory enzymes were affected by current, an experiment was run in which KCN was added to block oxygen uptake. He still found an increase in CO_2 formation when current was applied, indicating that anaerobic reactions were also affected by current. Experiments with various salt solutions, buffers, and carbohydrate depleted cells showed that the electrical stimulation of O_2 consumption was not due to pH changes. The respiration of cell lysates, made by freezing and thawing, was also increased by electrical pulses, indicating a direct effect on intracellular enzymes. However, an effect on the permeability of membranes, the plasma membrane of the intact cell, and/or the membranes of the interior particles such as mitochondria cannot be ruled out.

The effect of DC current on the budding of a red alga was studied by Schechter (1934). There was an increase in number of rhizoids produced with increasing currents over the range of 1 to 4 ma/cm^2. Plants with apexes toward the anode produce more rhizoids, and chromatophores tended to migrate toward the anode.

Osterhout (1950) noted effects of electric currents on unfertilized eggs of the marine worm Weris limbata. When an electric current was applied, minute granules just inside the surface of the egg absorbed water and swelled 300 times their original size. In this way a mass of jelly was produced that surrounded the egg with a zone about twice the diameter of the egg. With direct current the jelly first appeared on the cathode side. With alternating current it appeared on opposite sides facing the electrodes. If the current was shut off after one minute, the swelling continued. Alternating current was more effective than direct current.

In a communication Gillissen and Carlson (1952a) reported that antibiotic synthesis by an Aspergillus and a Penicillium specie in surface culture was hastened and enhanced by small AC currents. A more complete report appeared later by the same authors (1952b) in which some quantitative data were given. They showed that the synthesis of flavicin and kojic acid by A. oryzae approximately doubled when 18 ma of AC current was passed through the solution. The dry weight of mycelium was not appreciably changed. Penicillin production by P. notatum was approximately tripled with 10 to 44 ma of AC was used. They mentioned that neither the pH nor the temperature of the treated culture was significantly different from that of the controls and could not account for the effect.

Carlson (1954) continued his studies on the influence of low AC current on fermentations with some experiments on aerobic yeast growth. The current was applied to one liter of medium with carbon electrodes (1 cm in diameter, 7 cm long), and the aeration rate was 1.1 l/mu. Currents from 30 to 100 ma were used. The course of the fermentation was followed by measuring CO_2 production at 4-hour intervals. The total CO_2 production of the treated cultures after 12 hours was about 50% higher than that of the controls. At 36 hours, the difference in production between the control and treated cultures had decreased, but the treated cultures still produced about 15% more CO_2. Although similar results were obtained with both natural and synthetic media, the effects of the AC current were somewhat more dramatic with the latter. With synthetic media the stimulated culture used 25% more sugar than the control and produced about 33% more ethyl alcohol (5.8% vs. 4.3%). The final cell dry weight of the stimulated culture was only 5% higher than that of the control, and the final pH of the two cultures was the same.

The effect of AC currents (20-40 ma) on citric acid formation by strains of A. niger in surface culture was also studied by Carlson (1960a). The results were similar to those he obtained with yeast. Citric acid formation started earlier if current was applied to the culture. After 4 to 5 days the amount of citric acid in the treated culture was 30-100% greater than that in the control, depending on the strain of A. niger used. By the end of the fermentation, at 9 days, the differences in acid formation were less, but the amount of citric acid accumulated in the treated culture was still 10% higher. The final yields of citric acid based on the sugar used were 70.90 gm/liter or about 50%. Carlson also followed the formation of oxalic acid, a minor product yielding about 100 mg/liter. The yield of oxalic acid in the treated cultures was higher than the yield in the controls and followed the same relative pattern as found for citric acid.

The results were the same when an aluminum vessel was used instead of glass, and when aluminum or silver electrodes were used instead of carbon.

The volume of medium used in most of the experiments was 300 ml, but the author was able to scale up the process to 17 liters.

In the same year, Carlson (1960b) also published on the effects of AC currents on the biosynthesis of gluconic acid by A. niger in a submerged, aerated culture. Sugar, pH, and gluconic acid were measured throughout the course of the fermentations. Currents from 20 to 100 ma were tried, and he found 30-40 ma to be the optimum current range for enhancing gluconic acid synthesis. At 100 ma, metabolism was inhibited relative to controls. As he had found before, the effect of AC was most pronounced in the early stages of fermentation, 40-60 hours. As much as 100-200% more gluconic acid was formed with optimum amperages. By the end of the fermentation (90 hours), the treated cultures produced about 10% more fermentation products than the controls.

The pH of the fermentations was crudely controlled by adding calcium carbonate at intervals during the fermentation. Equal amounts of calcium carbonate were added to the treated and control cultures, but this usually resulted in a slightly higher pH (about 0.5 units) in the control culture because of lower gluconic acid formation. However, the pH levels of both the treated and control cultures were usually within the range 5.5 to 6.5, which was found to drop below 5.5, gluconic acid synthesis continued under the influence of current.

Electrically treated cultures did not retain their enhanced activity when transferred to a new medium, indicating that mutation did not occur. When the AC frequency was changed from 50 to 30 ma, 50,000 or 100,000 cps, fermentation again was accelerated, so that electrolytic manifestations probably did not account for the phenomenon entirely.

Wendel (1957) confirmed the work of Carlson on the stimulatory action of AC. He found that citric acid production by A. niger in surface culture was augmented when 15 ma was passed through the solution. In his 20 liter fermentations the citric acid yield was increased from 122 to 140 gm/liter, the latter figure corresponding to 80% of the sugar initially added, and oxalic acid production at 19 gm/liter was about 25% higher than without current. Wendel states that he has successfully used AC to enhance fermentative lactic acid production from cane molasses but did not give any data.

Rozenberg and Ulanovski (1960) measured the growth of bacteria

on cathodically polarized steel. At low current densities, less than 0.01 ma/cm^2, growth was inhibited; up to a current density of 0.2 ma/cm^2, growth was stimulated; at higher current densities growth decreased somewhat, but was still higher than in the controls. Their explanation was that at low current densities oxygen near the cathode was used up in a depolarization reaction and at higher current densities the pH in the vicinity of the cathode rose to about 9, which was better for growth than the initial pH of 7.5. However, this explanation could not be completely valid for B. megaterium, one of the cultures tested. This organism is a strict aerobe, and at higher current densities one would expect that no oxygen would be available, even though the pH might have been in a more suitable range.

Abood and his co-workers (1952, 1954, 1955) published a series of papers giving some insights concerning the effect of electrical pulses on metabolism. They found that glucose oxidation by rat brain homogenates, brain mitochondria, kidney homogenates, and liver mitochondria could be doubled by 16 volt electrical pulses. Oxidative phosphorylation in these preparations were inhibited at the same time. No effect was found on soluble enzyme systems such as ATP-ase, cytochrome oxidase, succinic dehydrogenase, hexokinase, and anaerobic glycolysis. They concluded that electrical effects were only manifested when biological membranes were involved.

Further experiments with brain mitochondria showed that ATP synthesis was inhibited, rather than its hydrolysis, and that the resulting increase in inorganic phosphate allowed a higher rate of glucose oxidation. The inhibition of oxidative phosphorylation was completely reversible, and shielding the electrodes with cosein did not alter the effect. They concluded that the effect did not occur at the immediate electrode surface, and that no permanent destruction or disorganization of the mitochondrial phosphorylating systems took place. Experiments with various substrates indicated that electrical pulses inhibited phosphorylation in the electron transport chain but not at the substrate level.

The distribution of Na^+ and K^+ ions in the mitochondria was changed by electrical pulses. Some reducing agents such as glutathione and sodium sulfide protected against the electrical effects, whereas other sources of the SH group such as cysteine did not. The authors suggest that the primary electrical effect is on the structural integrity of the mitochondria.

Krueger (1960) summarized the results of his investigation on the effects of gaseous ions on the cells of the trachial lining. He found that negatively charged oxygen ions increased the frequency of the cilia beating rate, but positively charged carbon dioxide ions inhibited it. Further, in vitro

experiments showed that negatively charged air ions increased the rate of succinate oxidation to fumarate by a pig heart homogenate. Also, negatively charged ions increased the rate of reoxidation of reduced cytochrome C by cytochrome oxidase. It is possible that ions such as these could be generated at electrodes by electrochemical reactions.

Hallum (1962) was recently awarded a patent for a method of altering a virus by controlled potential electrolysis. He found that the infectivity of virus particles was reduced by placing them in the anodic compartment of an electrolytic cell. If the applied voltage was less than 1.2 volt vs. calomel electrode, no attenuation took place. But higher voltages led to attenuation, so the rate of attenuation increased with anode voltage. At 1.6 volts the number of infective viruses was reduced by 1×10^{-8} in 3 hours. The treated particles retained their antigenic properties, and active vaccines were prepared from them. The author suggests that a specific oxidation of some molecule in the virus occurs at the anode which results in loss of infectivity, but that the overall structure of the particle is not changed sufficiently to alter its immunological properties.

Most recently Spohr (1964, 1965) has shown that bacterial growth, gas production from sewage, and B.O.D. reduction are accelerated when the bacteria are subjected to intermittent exposure to 60-cycle-45 ma currents. Lag periods were cut in half, and gas production from cellulose was more than doubled.

SECTION V

EXPERIMENTAL METHODS

Procedure for batch fermentation:

The apparatus (Fig. 1) consists of a specially designed shake flask fermentors, an electrical unit for current supply, platinum or carbon electrodes, and two rotary shakers. The fermentor is a 250ml. erlenmeyer flask with the top containing three openings and a side arm. Two of the three openings are used for electrodes which are connected to a current supply unit. The third opening is used to pass nitrogen gas through sterile cotton to maintain anaerobic conditions. A side arm on the flask is used for introducing an inoculum as well as taking the sample.

The fermentation apparatus is sterilized in an autoclave at 120°C for about 15-20 minutes. The synthetic medium is sterilized by filtration through sterilized membrane filters.

An inoculum flask is prepared by transferring the pure culture from a slant to the medium in a erlenmeyer flask. The flask is incubated on a shaker for 24 hours at 30°C for yeast and for 18 hours at 37-38°C for E. coli.

A series of fermentation flasks containing 150 ml. of sterilized media are inoculated from the inoculum flask. The flasks with no electrodes served as controls. AC and DC currents were used at known rates in the test fermentor flasks. For the yeast experiments growth was determined from the 4th to 12th hour after inoculation and for E. coli the growth curve was prepared by taking the readings from first hour through eighth hour. Approximately 5 ml. of sample was drawn, out of which 2 ml. was stored in a freezer for glucose analysis. In anaerobic fermentations the flasks are flushed with nitrogen after each sample is withdrawn. The temperature in the fermentation flask was recorded at the end of the fermentation period.

All concentration was measured turbidimetrically with a Klett-Summerson photoelectric colorimeter and a 660 mμ filter. The dry cell weight was calculated from a prepared standard curve. Glucose concentration was determined by an automated glucose analyzer.

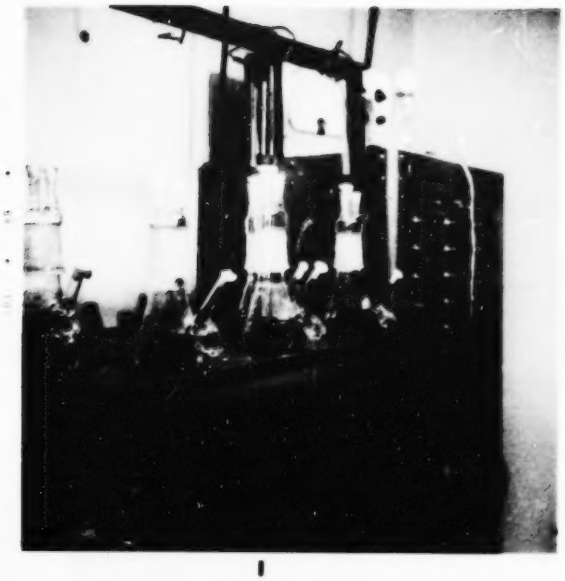


FIGURE 1. Batch Fermentation Experimental Apparatus
Note - power supply, culture flasks, carbon electrodes, shaker, and current meter.
The apparatus is placed in a constant temperature room.

Procedures for galactose transport studies:

The bacteria employed in this study was a derivative of E. coli ML30 which was designated ML 32,400. This was a galactose-negative strain obtained from B. L. Horecker.

To test the purity of galactokinaseless of ML 32,400 it was plated on a petri dishes which contained galactose as an only carbon source. No growth was found, indicating the culture to be pure.

The growth of ML 32,400 was carried out in a defined salt medium Horecker (1960) with a carbon source, such as succinate (pH = 7.0). ML 30, the wild type, was grown on the same salt medium plus galactose in order to adapt the cells to this sugar.

In these experiments the cells were grown aerobically at 37°C with shaking. The cells were harvested by centrifugation during the exponential growth (4 - 4 1/2 hr.) phase. Harvested cells were washed with medium and then resuspended in the same medium containing chloromycetin (50 µg/ml) to stop protein synthesis. The turbidity of cell suspension was measured optically with Klett-Summerson photoelectric colorimeter and a 660 mµfilter. The dry cell weight was calculated from a prepared standard curve. Approximately between 300 and 600 µg of bacterial dry wt. per ml. was used in each experiment.

A stock solution of galactose-C^{14} was prepared, and had a specific activity of 0.025 milicurie (2.78×10^6 cpm/ml). A calibration curve for concentration of galactose vs. cpm was made with Beckman liquid scintillation counter.

In most experiments, two sets of flasks containing ML 32,400 were grown. One was grown in presence of an electric field (120 ma-AC current) and another in absence of electric field and served as a control. After the cell suspension was prepared as described, about 18 ml of suspension was taken for experiment in a 50 ml erlenmeyer flask. The flask was shaken for about 20 mins to aerate the cells. To this suspension 1 ml of galactose-C^{14} stock solution and 0.1 ml of galactose-C^{12} 4×10^{-3} M solution were added. The contents of the flask were stirred with magnetic stirrer throughout the experiments.

One ml. samples were withdrawn from the flasks with a syringe at intervals of approximately 3 minutes up to the derivation of the experiment, about 40 minutes. The sample was filtered through a Gelman GA6 filter to separate the cells from the medium. The filter was then washed with

cold medium and put in a vial for later counting and estimation of the accumulation of galactose-C^{14}. Between 20 to 25 minutes after the start of the experiment, 1 ml of cold galactose-C^{12} 4×10^{-1} M was added to measure the exit reaction of accumulated galactose. The same type of experiments were performed with ML 30 to compare results.

In experiments to test the effect of electric fields on galactose permease synthesis during the growth cycle, 100 ml of medium, 1.0 ml of galactose-C^{14} stock solution and 0.1 ml of cold galactose-C^{12} 4×10^{-3} M solution were added initially along with 5 ml of inoculum. Samples were taken at half an hour intervals, filtered, washed and counted for galactose accumulation within the cells.

All radioactive samples were counted with a dioxane-based scintillation solvent mixture, which dissolved the filter material and formed a single phase. This solution was prepared by dissolving 5 to 6 gms ppo-2,5-Diphenyl-oxazole (scintillation grade) and 100 gms. of napthalene in 1 liter of Baker's Dioxane.

Background counts were measured with the filter pad alone, as well as after 1 ml of galactose-C^{14} stock colution was filtered through filter pad, and the pad wasked. To minimize background counts all soluntions used in the experiment were prefiltered and the filter pads were pre-soaked in distilled water.

Procedure for continuous culture fermentations:

The continuous fermentation system is shown schematically in Fig. 2. Photographic views are shown in Fig. 3. Media is filtered through the micro-filter and stored in sterilized media storage tanks. The sterile media is then pumped through a multiple metering pump to both the first and second stage fermentors where a continuous culture is maintained. In addition, product from the first stage fermentor may be pumped to the second stage fermentors. Note that the fermentors labelled "2A" and "2B" are in parallel mode. They each receive culture from fermentor number one as well as sterile media from the media storage tanks.

One of the features of this system is the variation of flow rates made possible by using the multiple metering pump. If the crossfeeds from the first to the second fermentors are set at zero, one may simultaneously generate three sets of single stage continuous fermentation data. Similarly, two sets of two stage fermentation data can be generated

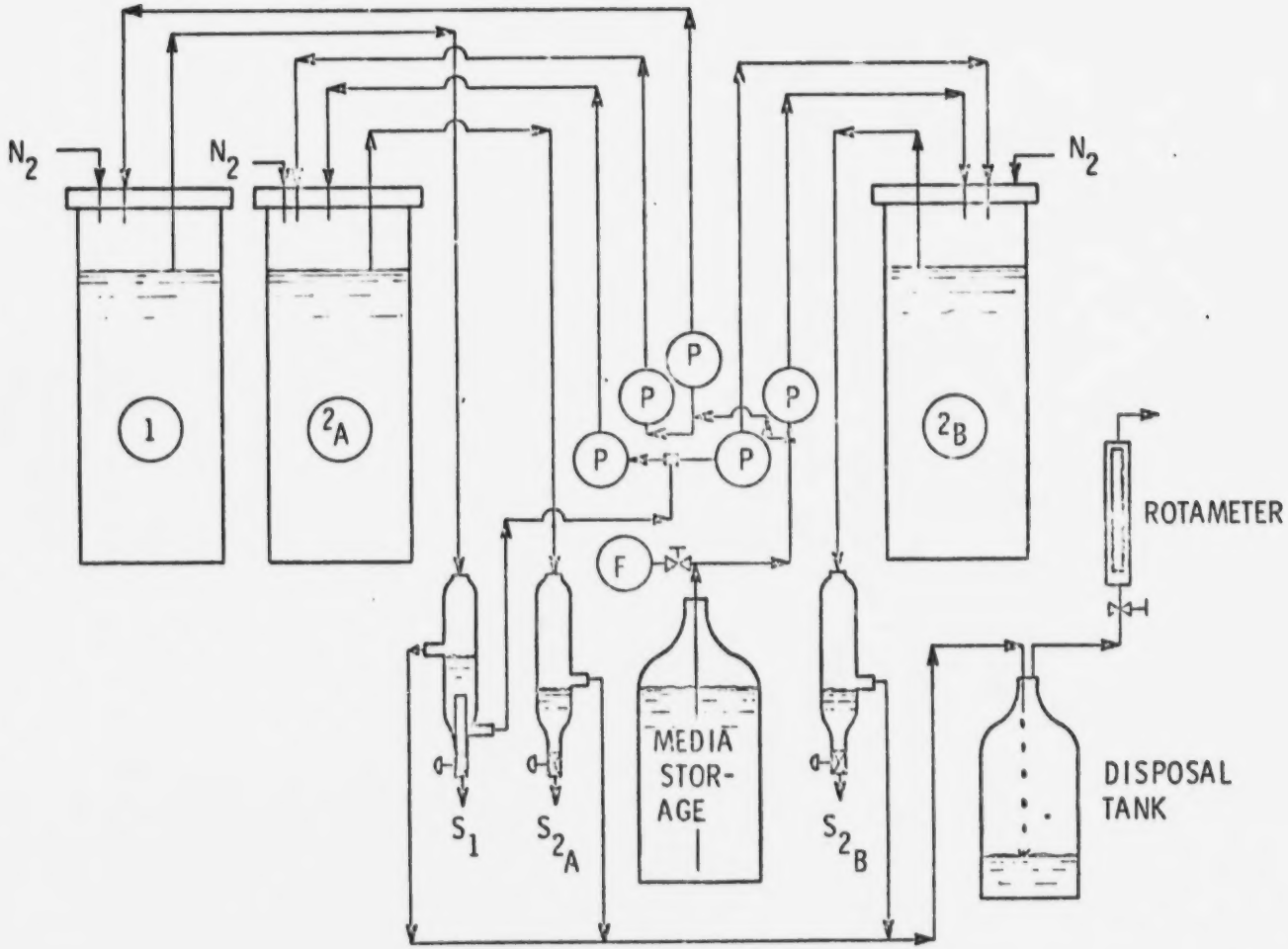


FIGURE 2. Equipment diagram for continuous culture unit.

Notes:

P ---One of the pumps of the Harvard Multiple Metering Pump

F ---The micro-filter

S_1, S_{2A}, and S_{2B}---Sample ports for fermentors 1, 2_A, and 2_B

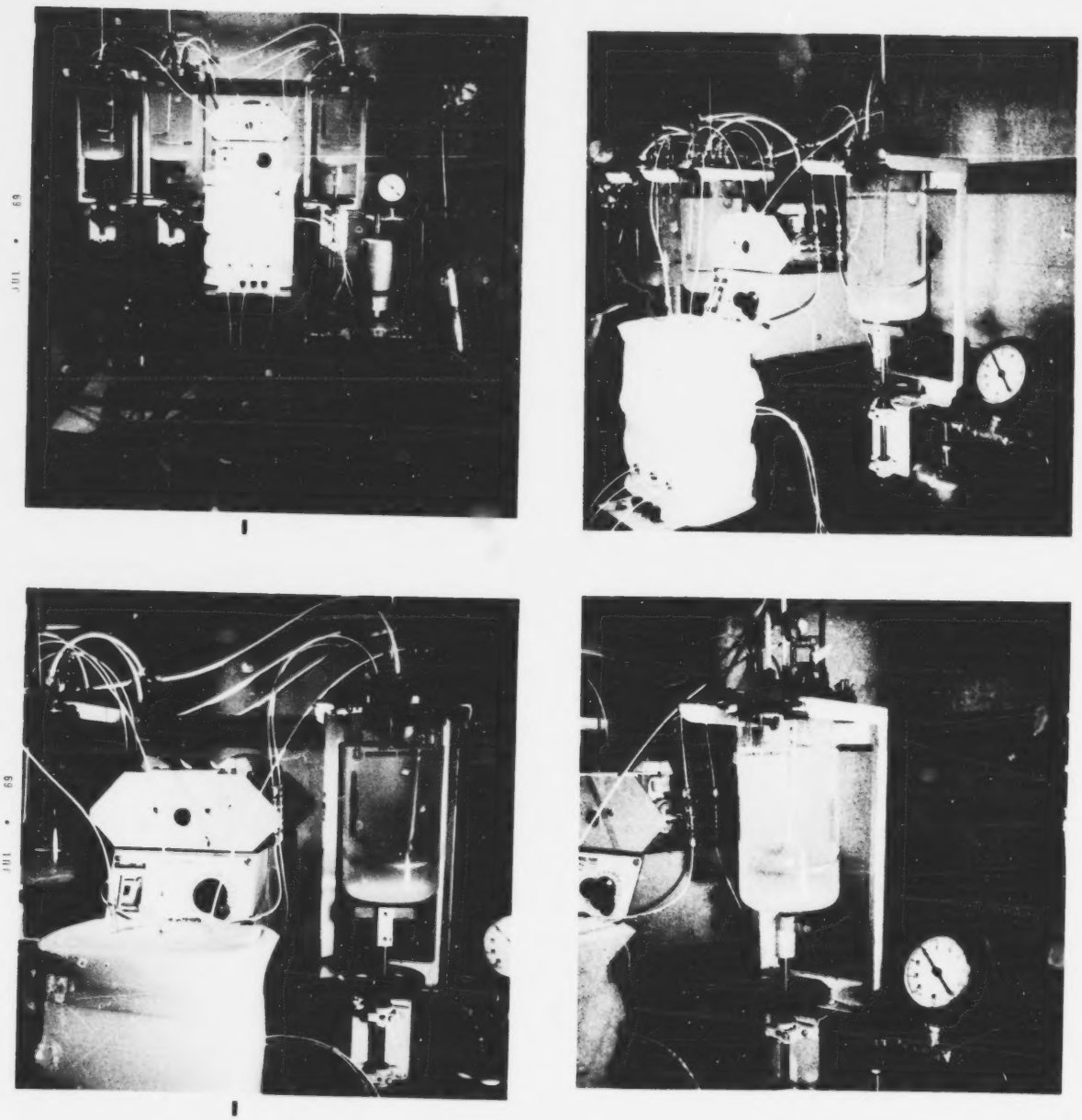


FIGURE 3. Four views of the continuous fermentation system. Note carbon electrodes in #2A

for each set of first stage conditions. In addition, if one sets the corresponding second stage flows at identical rates, he can operate "2A" as a "control" with "2B" as a "test" fermentor.

Other features include: a constant temperature bath with circulation to coils in each fermentor; magnetic stirring of all three fermentors from one variable speed drive motor; media storage tanks to allow several days operation before depletion of supply; provision to re-sterilize the filter apparatus and extend the experiment; solenoid operated sample valves; refrigerated sampling tubes and nitrogen sweep gas to remove gaseous growth products, to control the level in each fermentor and to reduce the possibility of contamination by maintaining a slight positive system pressure.

The sampling system was especially designed to collect representative samples and preserve them for analysis at a later time. The first part is a specially jacketed collection tube with a side arm to allow excess liquid to flow to the disposal tank. The second part is an automated sample holder with an electric timing system and a tray with tubes which are submerged in a refrigeration bath. The timing system operates solenoid valves on the bottom of the jacketed collection tubes, a two way solenoid valve system (which in one position exits the sample to the drain to wash the system and in the other position sends the sample to the sample tray), and the sample tray.

During the collection sequence, the system is first washed. Then the samples collect in the jacketed sample tubes for one minute. Finally the samples are drawn to the sample tray. Before the next sample time, the tray is moved to a new position. Samples drawn directly from the system are identical to those collected in this manner.

In the case of yeast, there is slight growth at even 2°C. Therefore, a chemical growth inhibitor was added to the sample tray tubes to eliminate even the slightest change in concentrations of glucose and cells.

This method of sampling allows continuous monitoring of the fermentation system without the need of a much more expensive and more complicated auto-analyzer system which would have to draw samples continuously from the fermentors. In addition, many different sampling intervals may be chosen at will.

In order to increase the amount of data generated during any experimental condition, three fermentors were operated in parallel mode for most of the experiments. Each

fermentor is set at a different dilution rate which spans from a low value (where nearly all the glucose is consumed) to a high value (where the cells are diluted so rapidly that wash-out is approached).

In order to assume that the ratios of dilution rates are constant, a Harvard Multiple Metering pump is used. This drives all three pumps from a single cam.

Levels are maintained by an overflow system. As a Nitrogen sweep flows through the fermentors it removes any broth which rises above the exit level.

The mixture of broth and nitrogen then passes to a set of sample collectors. The liquid sample collects in the bottom and the nitrogen exits out the side-arm. Any unused sample flows with the nitrogen to the disposal tank. The total nitrogen flow is controlled by the setting of a Rotameter on the exit stream from the disposal tank.

Samples may be drawn manually from the sample ports at the bottom of each sample collector. In addition, there is an automatic sample collection and storage system. This was designed in order to collect uniform samples at specified time intervals. The samples are treated to terminate all cellular growth and stored under refrigeration to prevent deterioration. This system was necessary in order to increase the quality of the results sufficiently to permit differentiation of the data for use in calculations of R_c and R_g (see equations 5 and 6).

Cell density is determined spectrophotometrically and glucose concentrations are determined by placing samples in an automated glucose analyzer which was built during this project. The glucose analysis is based on the Potassium ferricyanide oxidation of the remaining glucose.

Each fermentor has been coated with a special silicone treatment to avoid the sticking of growing cells to the walls. The fermentors are kept "well-mixed" by a system of magnetic stirrers and temperature is controlled by a constant temperature circulation system.

The electrical equipment consists of the electrodes, the amplifier and the signal generator.

The electrodes are an electrode grade carbon rod which has been enclosed in pyrex at one end using epoxy glue for the sealer. This assumes that no metals can form electrolysis products in the broth. The rods are 1/4 inch in diameter and 2 inches long. They are positioned in the

middle of the fermentors at a separation of 2 3/8 inches.

The amplifier is a Stromberg-Carlson power amplifier which is capable of delivering a maximum of 100 watts RMS. This also serves as an isolation transformer.

The signal generator is a Wavetek voltage controlled generator. It is capable of generating sine, ramp, saw tooth or square waves at frequencies from 10^{-2} to 10^{6} cycles/second.

For direct current studies, an Electronic Measurements Co. D.C. Power supply capable of delivering 20 volts at 1 amp was used.

A defined glucose media based on Olson and Johnson was selected and the numbered strain Saccharomyces cerevisiea LK2G12 was chosen. Anaerobic fermentation was picked to eliminate the difficulty of maintaining oxygen concentrations uniform. Temperature was controlled at 32 °C. The fact that yeast ferments at lower temperatures and pH's than many contaminants and is not toxic were some of the reasons for choosing it.

The general experimental procedure is outlined below:

1. Withdraw about 5 ml of cold liquid samples. Terminate growth and hold in cold storage for analysis. Record time.

2. Measure the turbidity with the Klett-Somerson spectrophotometer. Record the scale readings.

3. Place samples in the automated glucose analyzer. Record the standard curve readings before and after each set of samples. Record readings for each sample.

4. Punch the raw data, calibration curves, and coded conditions of the experiment on IBM cards as input to an analysis program which utilized the IBM 360-67 model digital computer.

5. The computer program performs the following operations:
   a. Calculate the cell concentrations in mg/ml.
   b. Calculate the glucose concentrations in mg/ml. Also correct for any drift in the automated analysis system.
   c. Calculate dC/dt and dG/dt at each time interval during the run. The method employs a polynomial smoothing of the data.

d. Calculate R_c, $R_q = \mu$, and Y from equations four, five, six, and eight (above).
e. Attempt to "fit" the calculated values of μ to the various models which have been proposed through use of a least squares regression technique.

SECTION VI

RESULTS AND DISCUSSION

Effect of electric fields in batch fermentations

Typical growth curves for batch shake flask culture are shown in Fig. 4. Because of volume restrictions, samples could not be taken at more frequent than hourly intervals and the growth curves are not smooth. An average estimate of stimulation was calculated in the following manner: during the rapid growth phase (6 to 10 hours for yeast) the difference in cell concentration between test and control cultures divided by the cell concentration for the control was computed. The average of these numbers, expressed as percent, for the growth period was considered the effective stimulation. The effect of DC current on anaerobic yeast growth is shown in Fig. 5. Each point is the average of about 5 runs. Increases in growth rate of about 10 percent are about average. The bars on this graph indicate the total spread in the data and point out the difficulty in obtaining highly reproducible results in batch experiments. Glucose utilization was also measured to see if it paralleled the growth rate. The results in Fig. 6 show that DC currents increased glucose utilization to a lesser extent.

The results of about 50 experiments with AC currents and anaerobically grown E. coli are shown in Fig. 7. Here again a moderate stimulation is exhibited, but at much higher currents. It was found that most of the AC power was dissapated as resistive losses in the solution, causing a small rise in the temperature of the flask. The temperature rise was normally less than 1°C even at the higher currents. Growth experiments were run over a wide temperature range to evaluate the effect of this variable. The results shown in Fig. 8 (expressed as percent stimulation relative to a 37°C control culture) indicate that a 1°C temperature rise could account for about 8% growth stimulation. In Fig. 7 the maximum increase in growth rate with AC current is about 16% indicating that about half the growth increase could be attributed to the applied electrical current. In Fig. 9 the increase in glucose utilization is even greater.

In summary, AC and DC currents seem to increase the rate of cell growth and glucose utilization. The effect may be on the order of 5-10% increase in rate, but the exact magnitude is difficult to assess because of the great variability in batch fermentation experiments even where controls are included in each experiment. The continuous culture apparatus allows a more sensitive and stable evaluation of the effects of electrical currents.

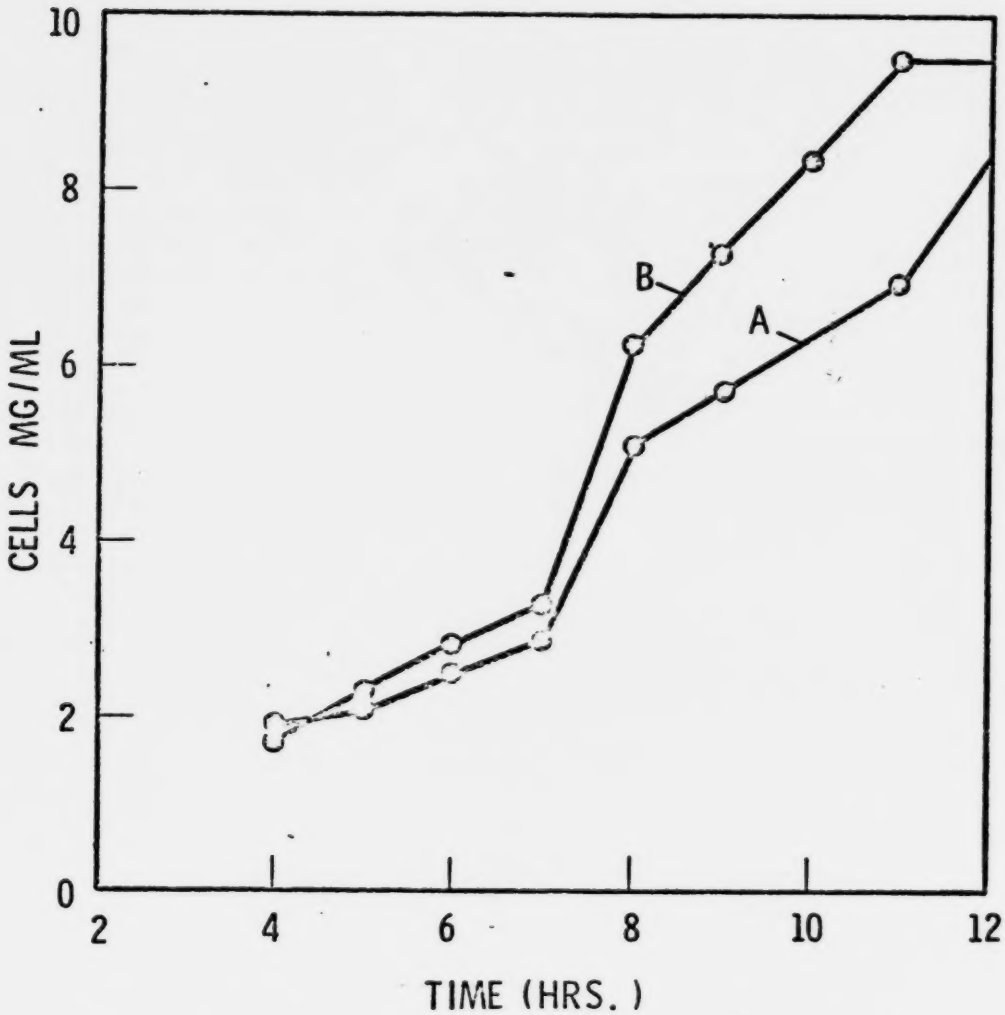


FIGURE 4. Typical growth curves of control and stimulated cultures of yeast.

A. Control Flask

B. Test Flask 0.2ma DC current applied

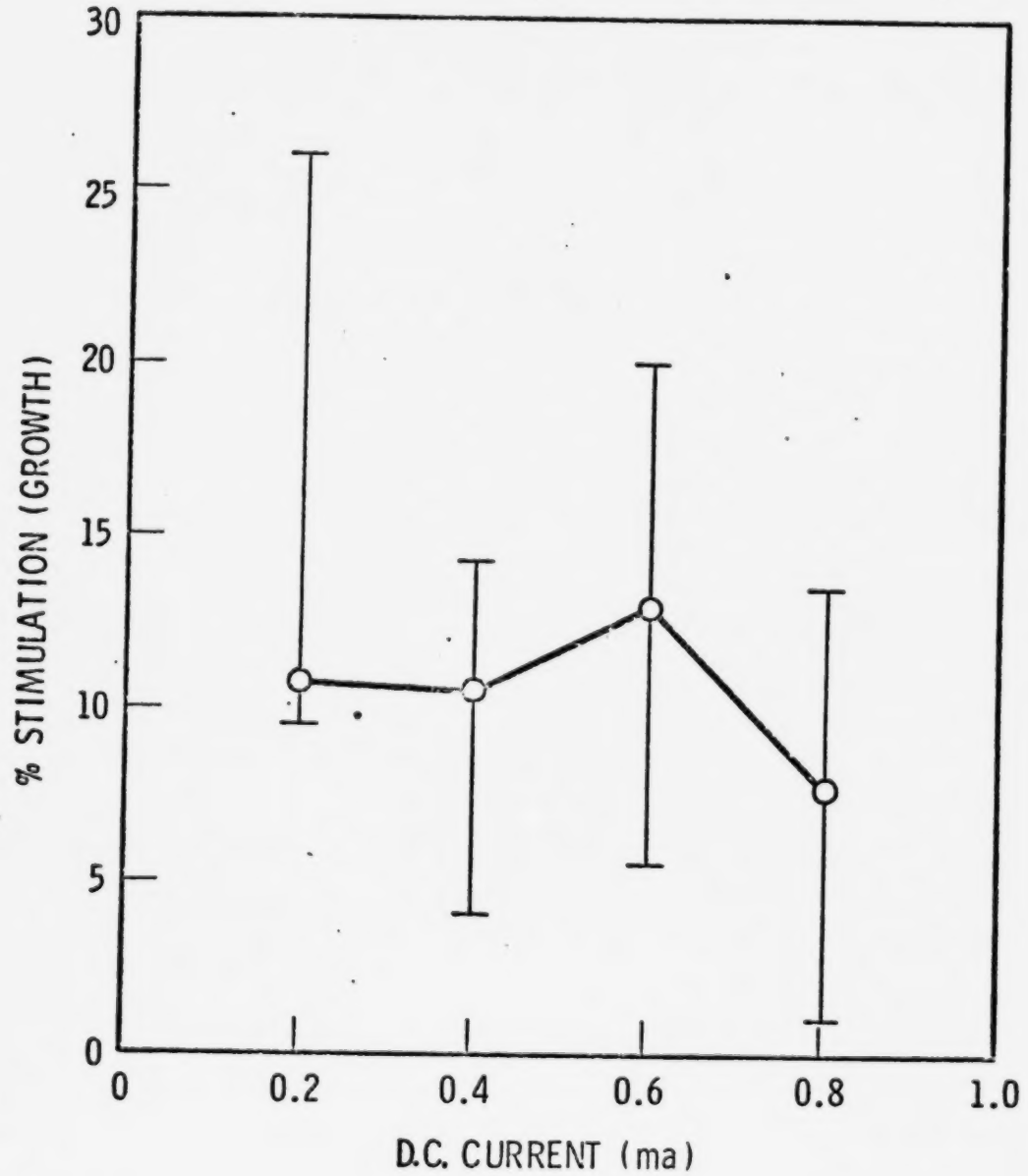


FIGURE 5. Stimulation of yeast growth as a function of applied direct current. Bars indicate spread of data.

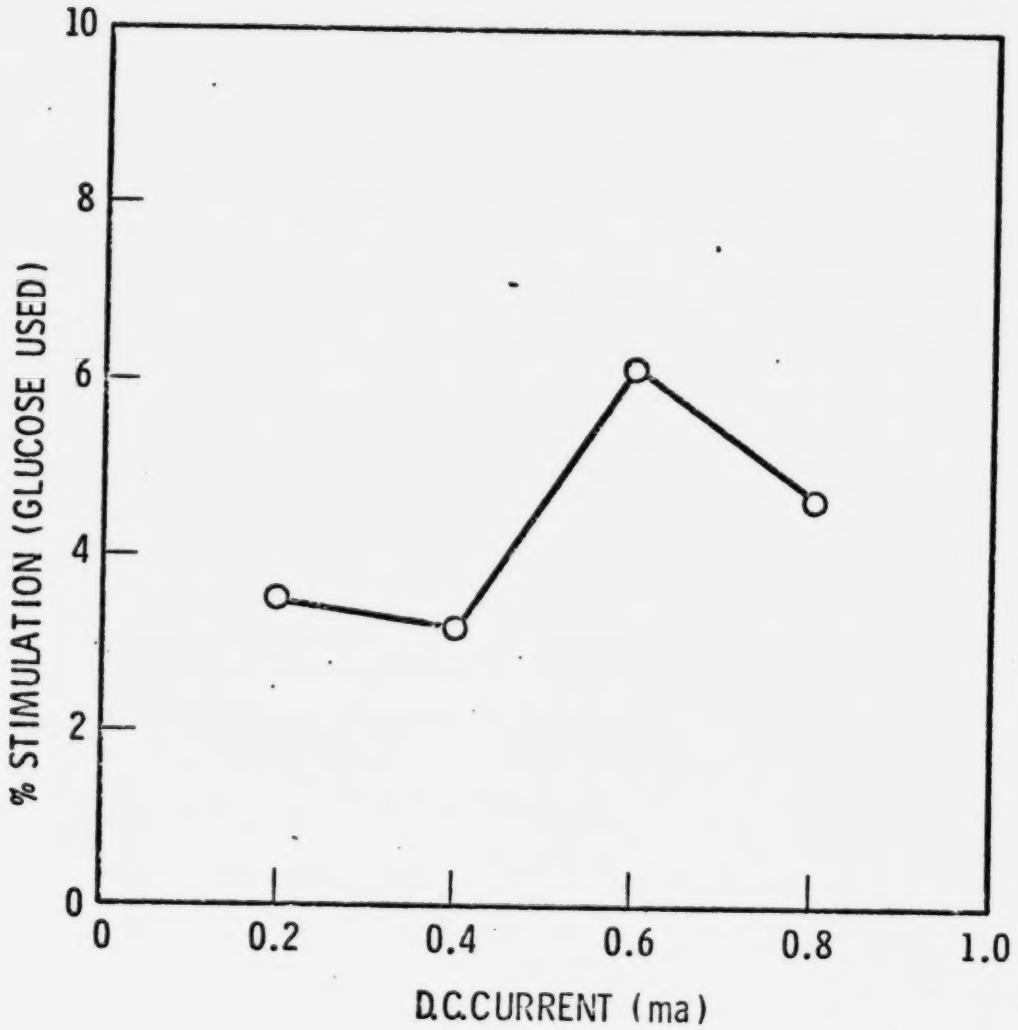


FIGURE 6. Stimulation of glucose utilization as a function of applied direct current.

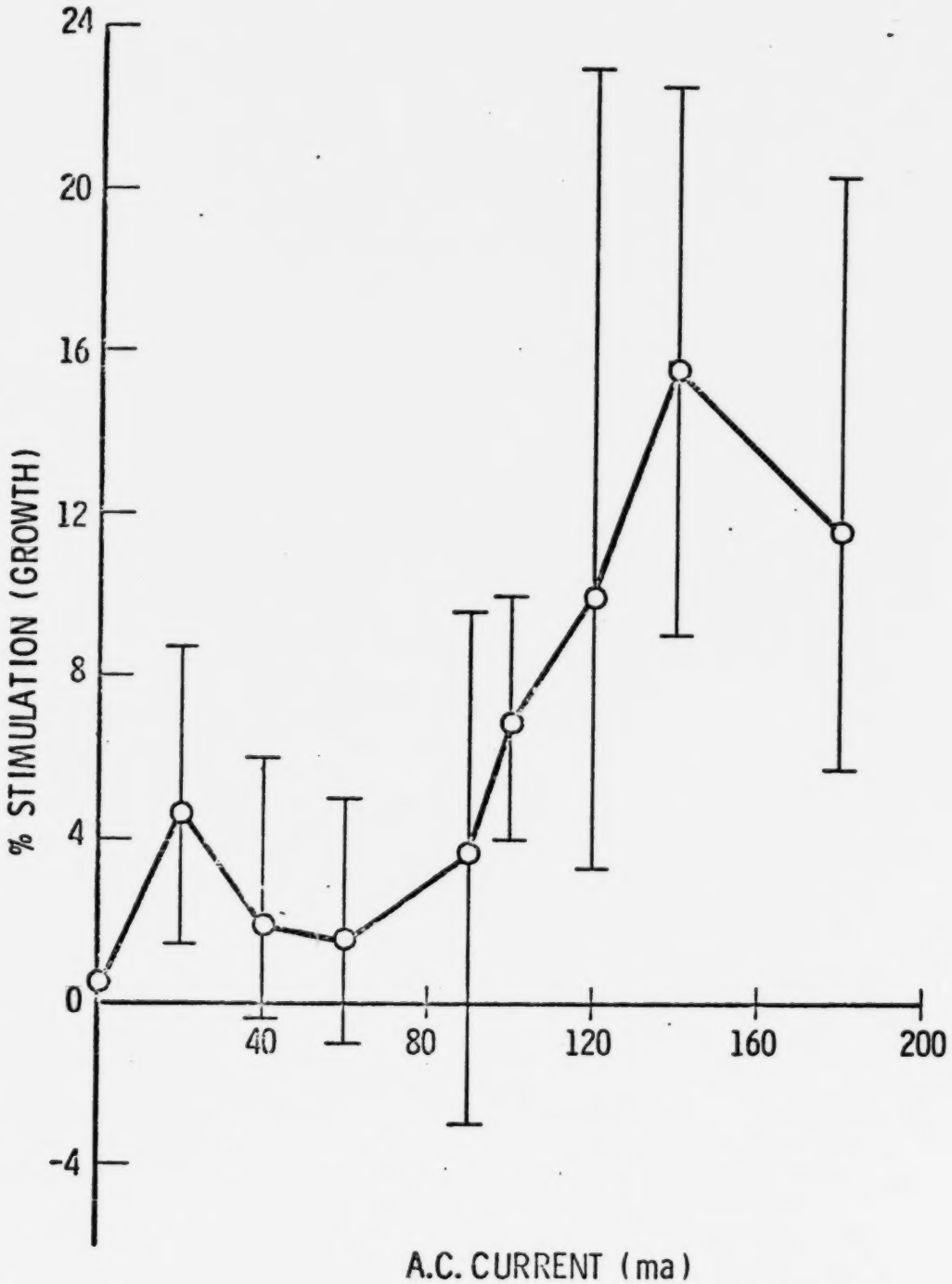


FIGURE 7. Stimulation of anaerobic growth of E. coli by applied alternating current. Bars indicate spread of data.

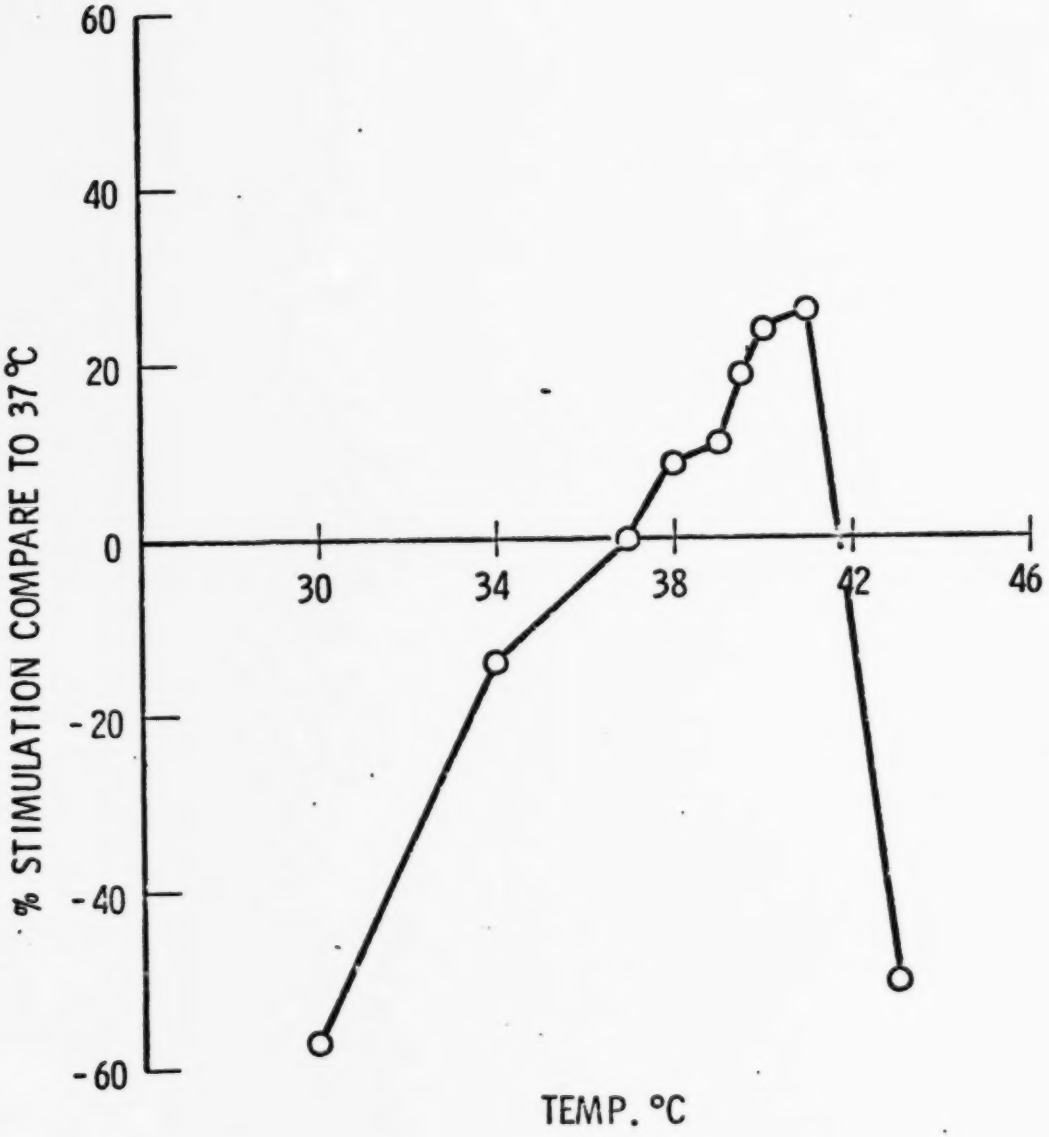


FIGURE 8. Effect of temperature on the growth of E. coli anaerobically. Growth rate at 37°C taken as reference condition.

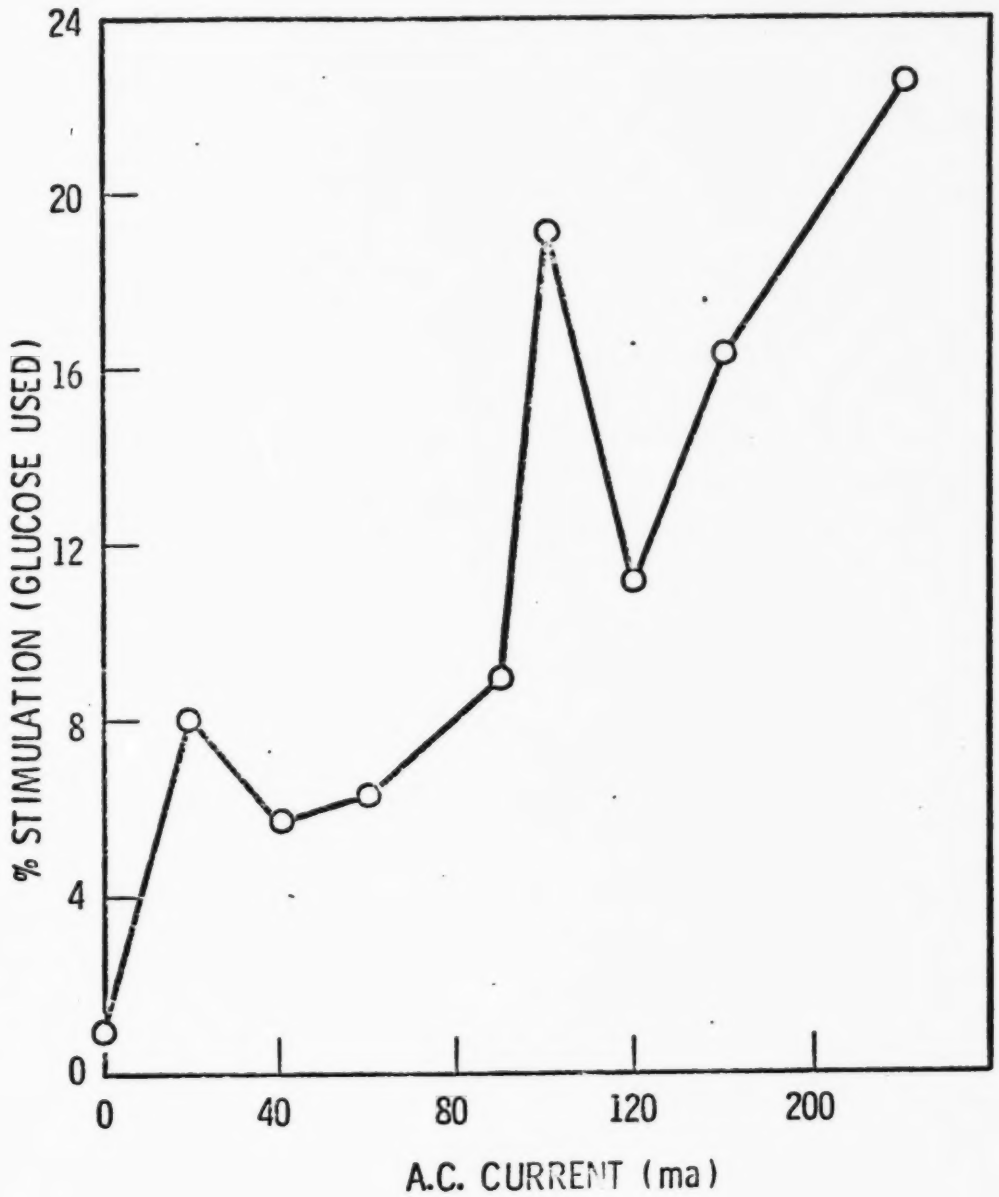


FIGURE 9. Stimulation of glucose utilization by E. coli as a function of applied alternating current.

Effects of electric fields in continuous fermentations

The initial experiments which were with no electrical stimulation show that certain models for the specific growth rate (μ) are significantly better than others Figs. (10, 11). Both the Monod model and the Contois model give accurate predictions for steady-state or small deviations from steady-state. None of the models is capable of handling large transients.

Each of the experiments begins with inoculation of the fermentation flask with an 18 hour batch culture. The response of the system as it approaches a steady-state seems almost impossible to predict with the Monod or Contois forms. This may be due to the fact that each of these models actually apply to bacterial growth. At any rate, none of the electrical experiments caused much deviation from the control steady-state conditions.

The electrical experiments were mostly confined to DC, sine wave, and ramp wave. Two conditions of square waves were also tried. The Monod form is used to report the result of electric current on the growth rate. The Monod constants are determined by least squares computer analysis. Each experiement is reported with its own controls rather than attempting to determine average control conditions. This is necessary since there seems to be a slight unexplained difference in growth rate from experiment to experiment.

TABLE 1

DIRECT CURRENT

Current	Voltage Applied	μ_m †	K_s †
Small	0	.33	.04
	0	.32	.08
	.2	.34	.05
	.5	.28	.04
	.9	.30	.03
1 ma	1	.31	.04
1.3ma	1.35	.33	.10
1.9ma	2	.33	.05
4.3ma	2.5	.30	.07
12 ma	3	.24	.05

† See next page for explanation.

Experiments at 3.5 and 5 volts were also carried out, but here growth was inhibited and it was possible to correlate using the Monod or Contois forms, since the decrease in growth rate was very pronounced. At these higher voltages electrolysis was definitely taking place at the electrodes.

TABLE 2

RAMP WAVE (AC)

Voltage Applied	Frequency (cps)	μ_m †	K_s †
0	0	.37	.27
0	0	.35	.06
15	10	.36	.08
15	30	.36	.11
15	75	.34	.06
15	200	.35	.07
15	450	.35	.08
15	1,000	.31	.03
* 15	2,000	.29	.03
15	10,000	.33	.04
15	20,000	.30	.02
25	1,000	.32	.05

* Bad correlation according to statistical analysis. All voltages are RMS

† The data were fitted to the Monod growth equation,

$$\mu = \frac{\mu_m S}{K_s + S}$$

where μ = specific growth rate constant (hr^{-1})

μ_m = maximum specific growth rate constant (hr^{-1})

S = substrate (glucose) concentration (gm/ℓ.)

K_s = model constant (gm/ℓ.)

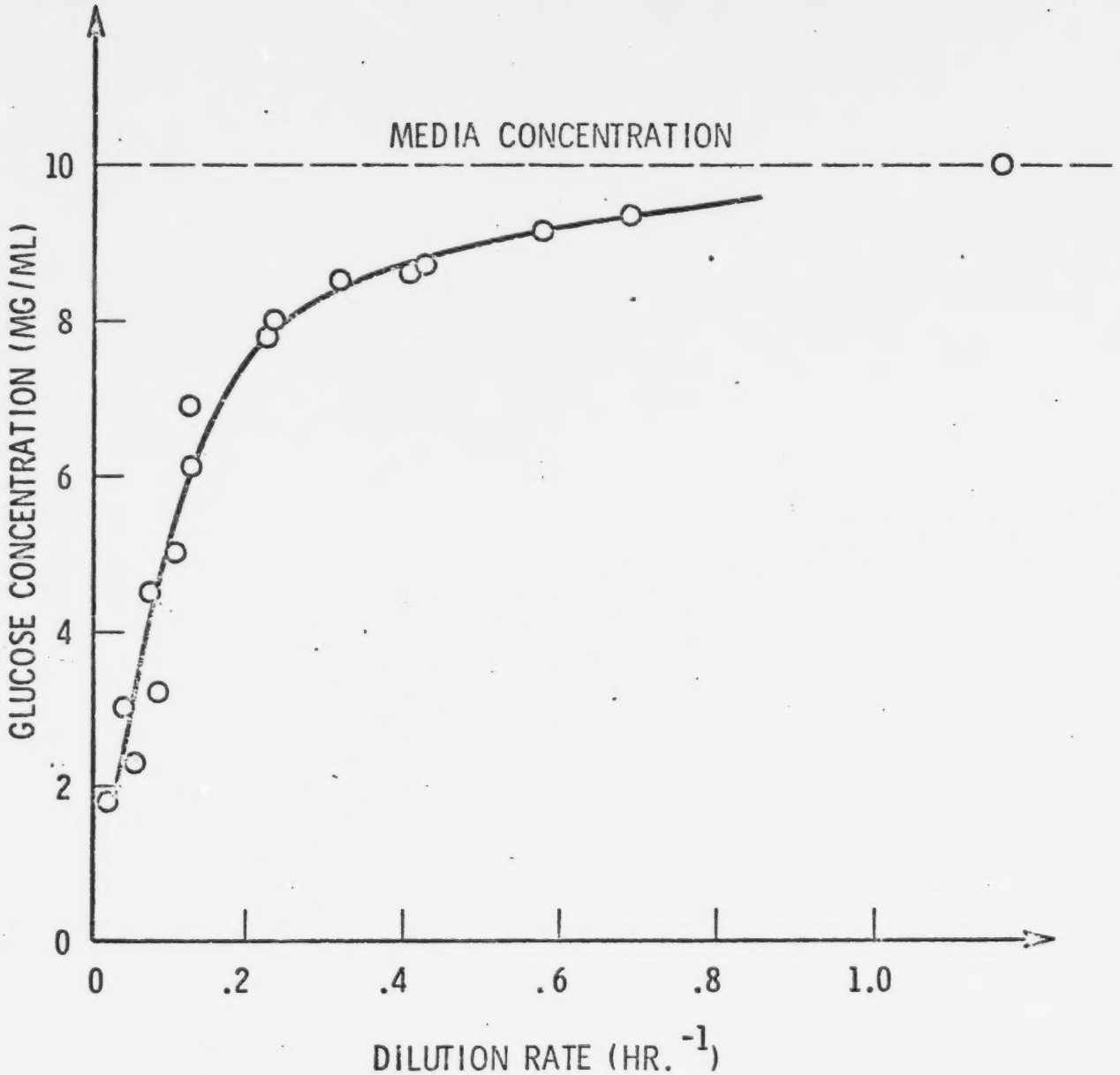


FIGURE 10. Substrate Concentration vs. Dilution Rate for Single Stage Fermentation. S. C. LK2G12 @ 30°C

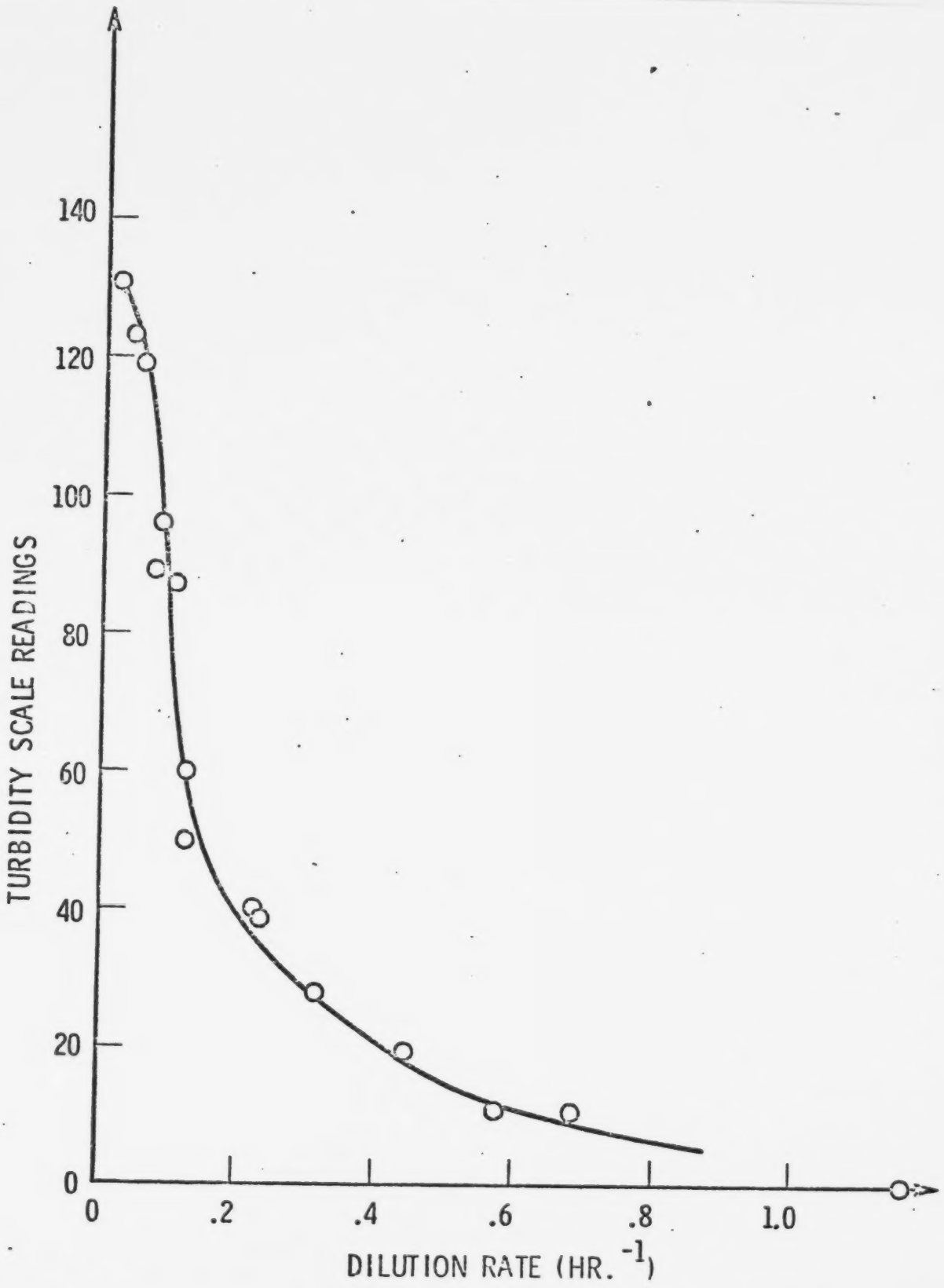


FIGURE 11. Turbidity Readings vs. Dilution Rate for Single Stage Fermentation. S. C. LK2G12 @ 30°C

TABLE 3

SINE WAVE (AC)

	Voltage Applied	Frequency (cps)	μ_m	K_s
	0	0	.31	.07
	15	20,000	.33	.04
*	15	10,000	.25	.03
	15	5,000	.33	.07
	15	2,500	.30	.03
	20	8,000	.33	.05
	20	16,000	.29	.03
*	15	16,000	.34	.16
	0	0	.34	.09
	15	30,000	.26	.03
	0	0	.31	.04

* Bad correlation according to statistical analysis.

In addition to these results, there were several runs in which lower frequencies were tried. These were all performed at a single intermediate dilution rate of $\sim$.25 hr^{-1}. The glucose and cell concentrations were measured, but calculation of the Monod or Contois model constants was not possible. 60 cycle runs of 10, 15, 20, and 25 volts were carried out early in the experimental phase of the work. The lower three showed no trends from the previous steady-state values. The 25 volt run had problems with temperature control and gave meaningless results. In similar experiments at 15 volts and 18,000 cps, 10 volts and 60 cps, 10 volts and 75 cps, and 15 volts and 1,000 cps there was no noticeable trend in glucose or cell concentrations.

The two square wave experiments were carried out at 1,000 cps and 15 and 25 RMS volts. These also gave no surprising results. The Monod constants were μ_m = .35 and .33 and K_s = .10 and .08 for the 15 and 25 volt experiments respectively.

In summary, DC voltages below 2 - 2.5 have no significant effect on the growth of yeast. Above this level, growth falls off significantly. In order to be significant, one would expect at least a 10% change in μ_m. For alternating current sine and ramp waves ther is no significant difference

between controls and experiments with the exception of a decrease in growth rate which appeared in the 30,000 cps sine wave experiment.

Effect of electric fields on transport through microbial cell membranes

In order to evaluate the effect of electric fields on transport into microbial cells a mutant strain of E. coli was selected which does not metabolize galactose, but has a specific transport "permease" for this substrate

We first checked the effect of electric fields on the aerobic growth of the E. coli (ML 32,400) mutant which is incapable of metabolizing galactose. These tests were conducted in batch flask culture, just as the previous anaerobic growth of yeast. Growth studies were made over a wide range of currents. A typical example of the results obtained in this type of evaluation is shown in Fig. (12). The maximum stimulation on growth rate is shown during the rapid log phase. In this phase cell growth rate in an A.C. field was about 40% greater than the corresponding growth of a control culture at the same cell concentration. However, the stimulation in growth was not reproducible in magnitude but usually was positive in nature.

A check of the efficiency of the counting technique for galactose-C^{14} was made as a function of galactose concentration to see if quenching or self adsorption was significant. The results given in Fig. 13 show that up to a level of about 0.007 μ moles of galactose in the counting vial, no corrections are needed.

Another test of technique was made with the wild-type E. coli (ML 30). This culture should accumulate galactose linearly with time, without becoming saturated, because the wild type can metabolize galactose. This behavior seems to be occurring under our conditions, as shown in Fig. 14. A similar experiment, but with the mutant (ML 32,400) Fig. 15 shows saturation of the cells with galactose after about 30 minutes. Presumably, at this time influx into the cells via the active galactose permease entrance system is balanced by the exit processes of the cell.

Next, the reversibility of the transport system in the mutant was checked by adding excess non-radioactive galactose to the cell suspension after 20 minutes. The accumulated radioactivity in the cells was displaced by the added galactose(Fig. 16) indicating that the galactose was not

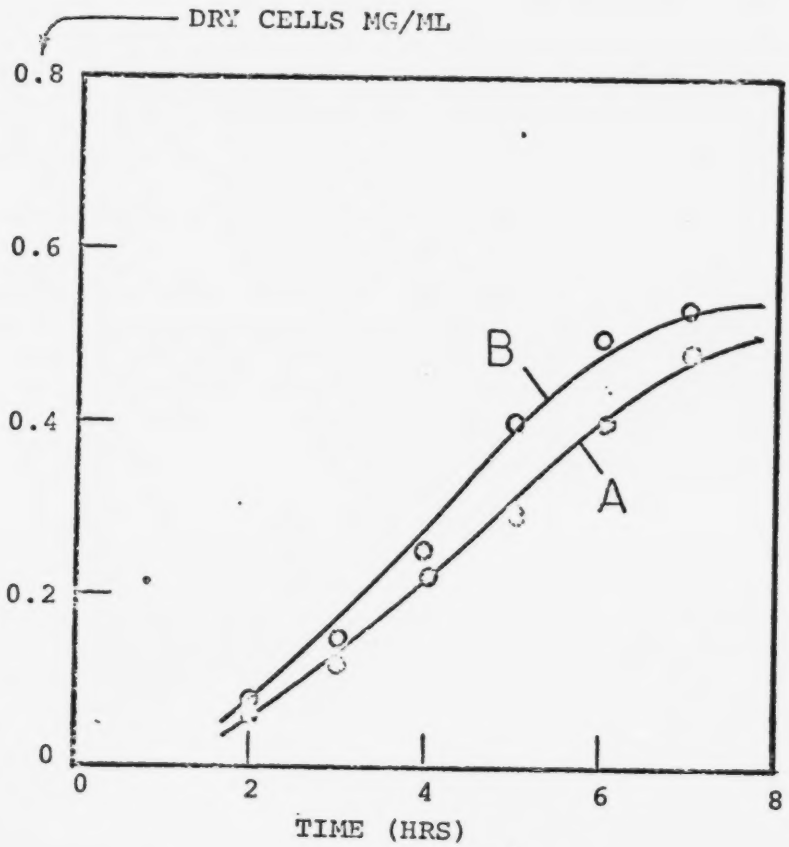


FIGURE 12. Effect of applied AC current (120 ma) on aerobic growth of E. coli ML 32,400

A. Control flask
B. Test flask

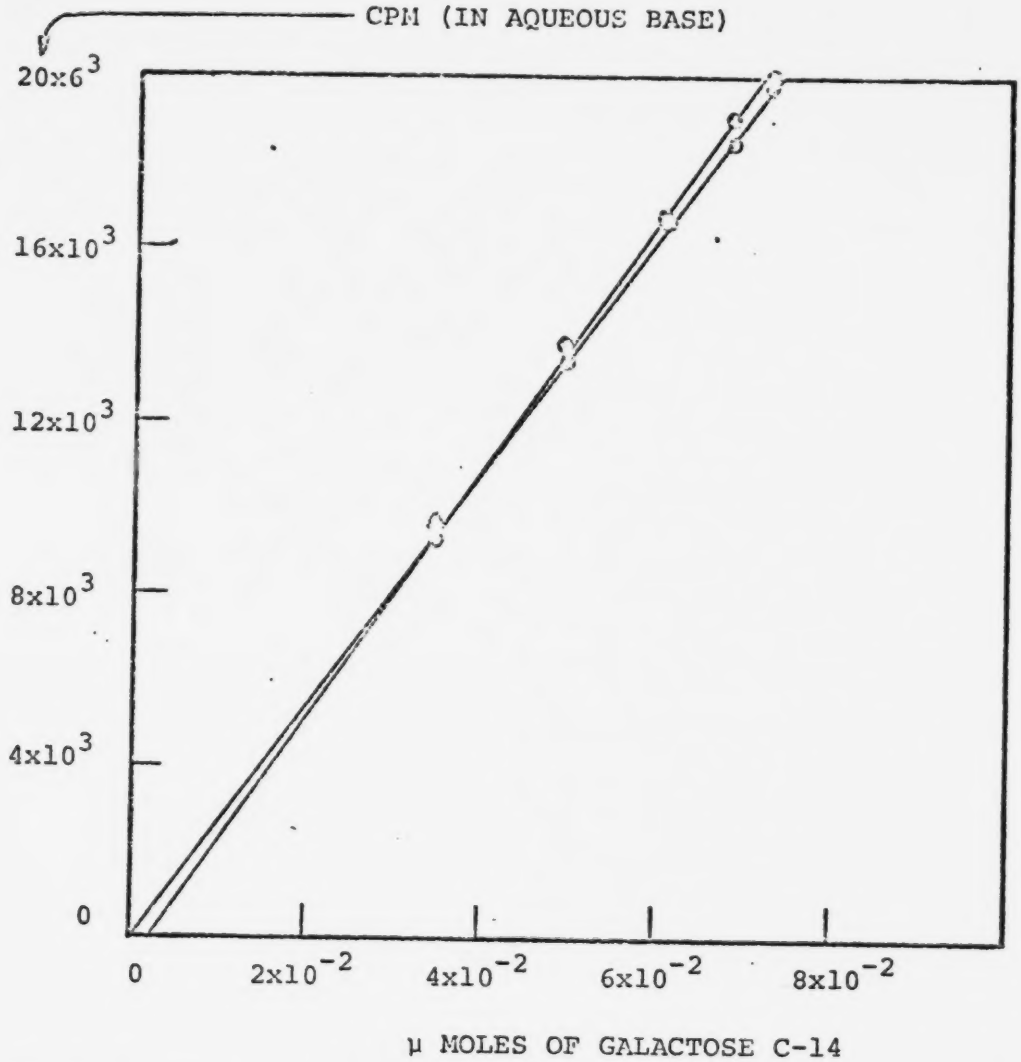


FIGURE 13. Calibration of galactose counting procedure

- • Expected counts
- ∘ Actual counts

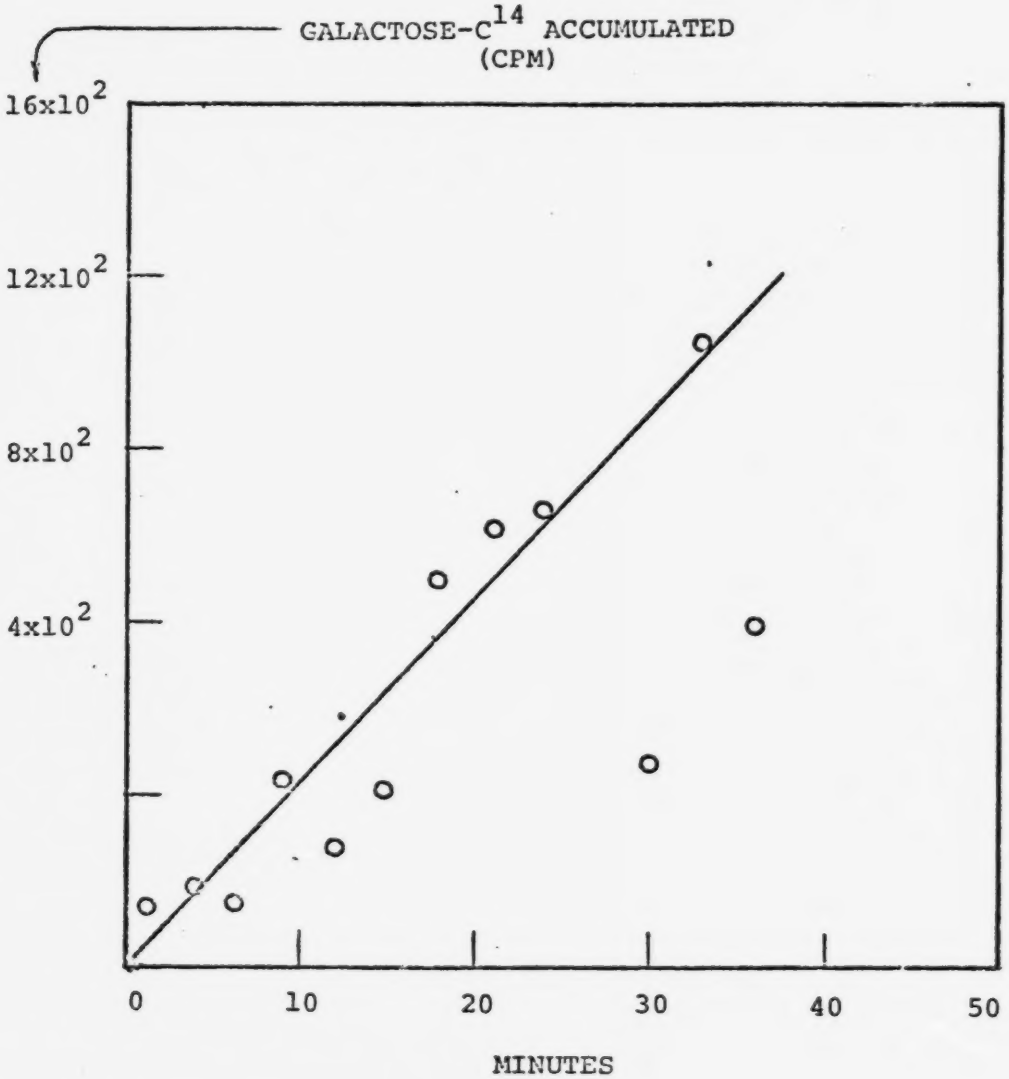


FIGURE 14. Transport and accumulation of galactose by a wild type E. coli. At zero time a washed suspension of succinate-galactose grown cells of E. coli ML 30 containing 0.625 MG of bacterial dry wt per ML was treated with a solution of galactose-C^{14}.

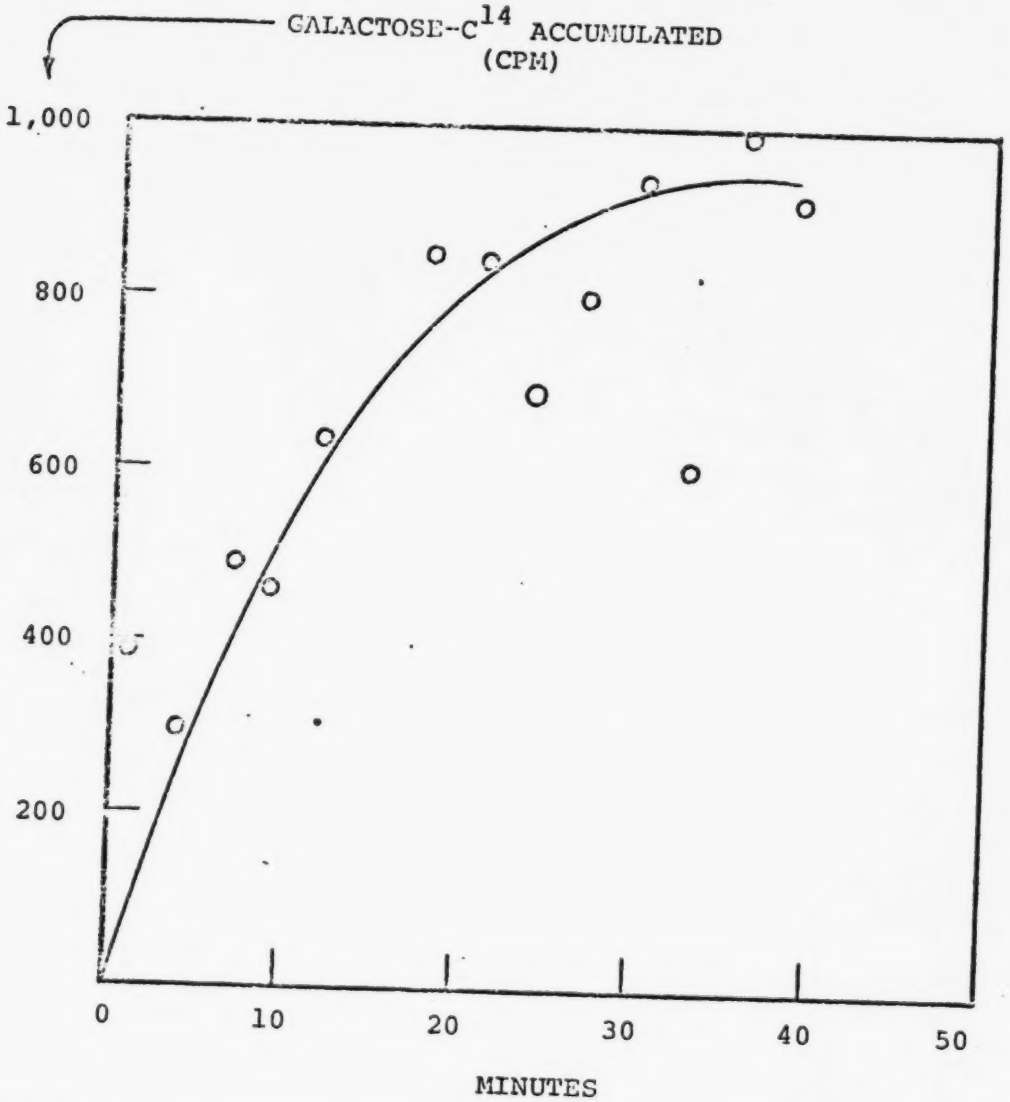


FIGURE 15. Transport and accumulation of galactose by a galactokinase - less mutant of E. coli. At zero time a washed suspension of succinate-grown cells of E. coli ML 32,400 containing 0.600 MG of bacterial dry wt per ML was treated with a solution of C^{14}-galactose.

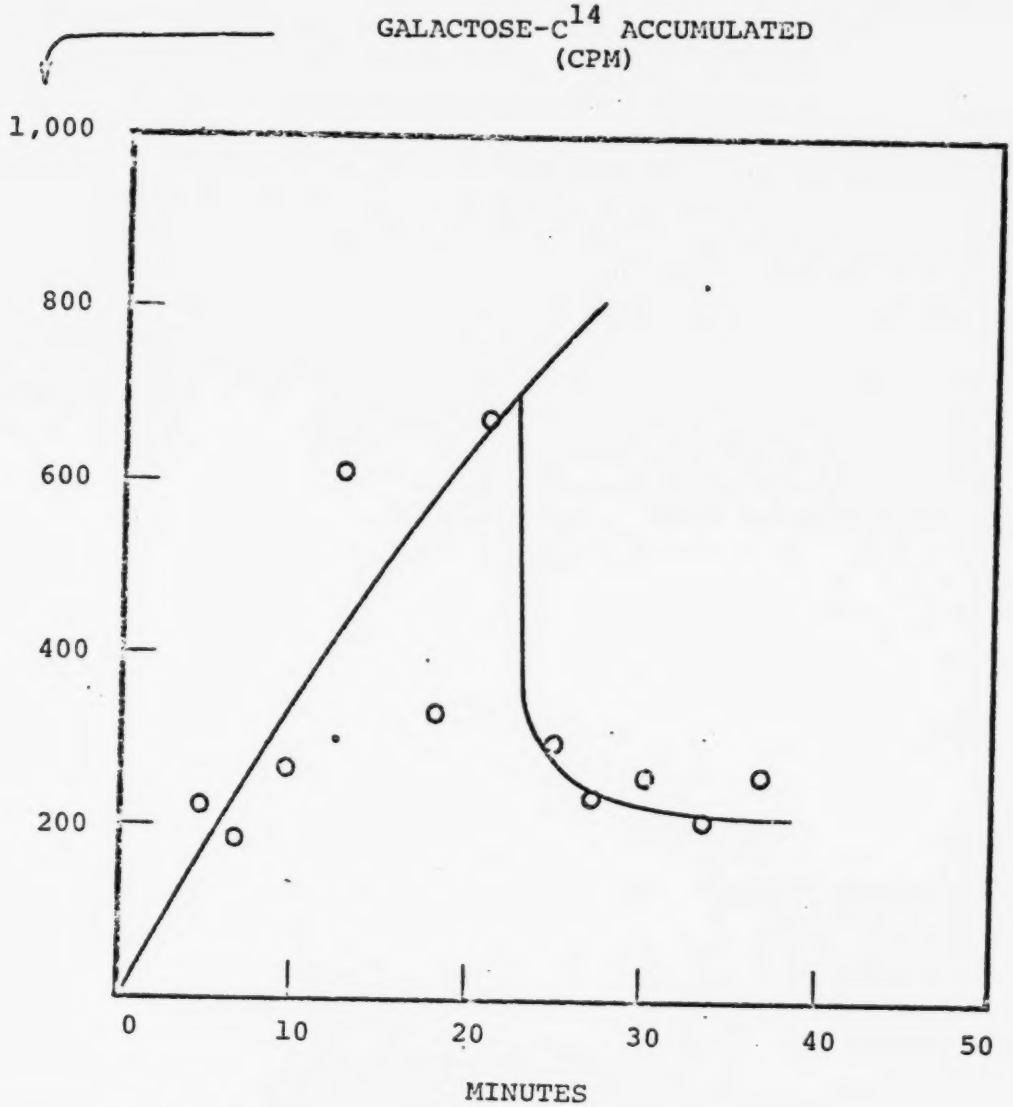


FIGURE 16. Reversibility of galactose transport systems. At zero time a washed suspension of succinate-grown cells of E. coli ML 32,400 containing 0.625 MG of bacterial dry wt per ML was treated with a solution of galactose-C^{14}. After 22 minutes the accumulated radioactive sugar was displaced by the addition of excess "cold" galactose.

involved in irreversible reactions. However, the data were very scattered, and it is difficult to fit a simple curve to the data.

Finally, many experiments were devoted to comparing the galactose transport rate of mutant E. coli cells with and without the exposure to electric fields during the growth phase of the cells. A typical result is shown in Fig. 17. Here it can be seen that the mutants grown while exposed to an electric field (60 cps, 120 ma) accumulate more galactose over a 20 minute period than cells not exposed to an electric field. The rate of transport appears to be greater, indicating an increase in synthesis of the galactose permease system by electric field. Again, in these tests the data were very variable, and did not lend themselves to a kinetic analysis.

Another attempt to demonstrate the effect of electric fields on biological transport rates was made by growing mutant cells in the presence of radioactive galactose and measuring accumulated radioactivity within the cells as a function of time. It appears (Fig. 18) that slightly higher levels of galactose are accumulated by cells exposed to electric fields in the first few hours of growth.

The balance of the information obtained by these studies on a very specific membrane transport system are indicative of a positive effect of electric fields on transport into microbial cells. However further work is needed to reliably establish the magnitude and kinetics of this stimulatory action of electric fields.

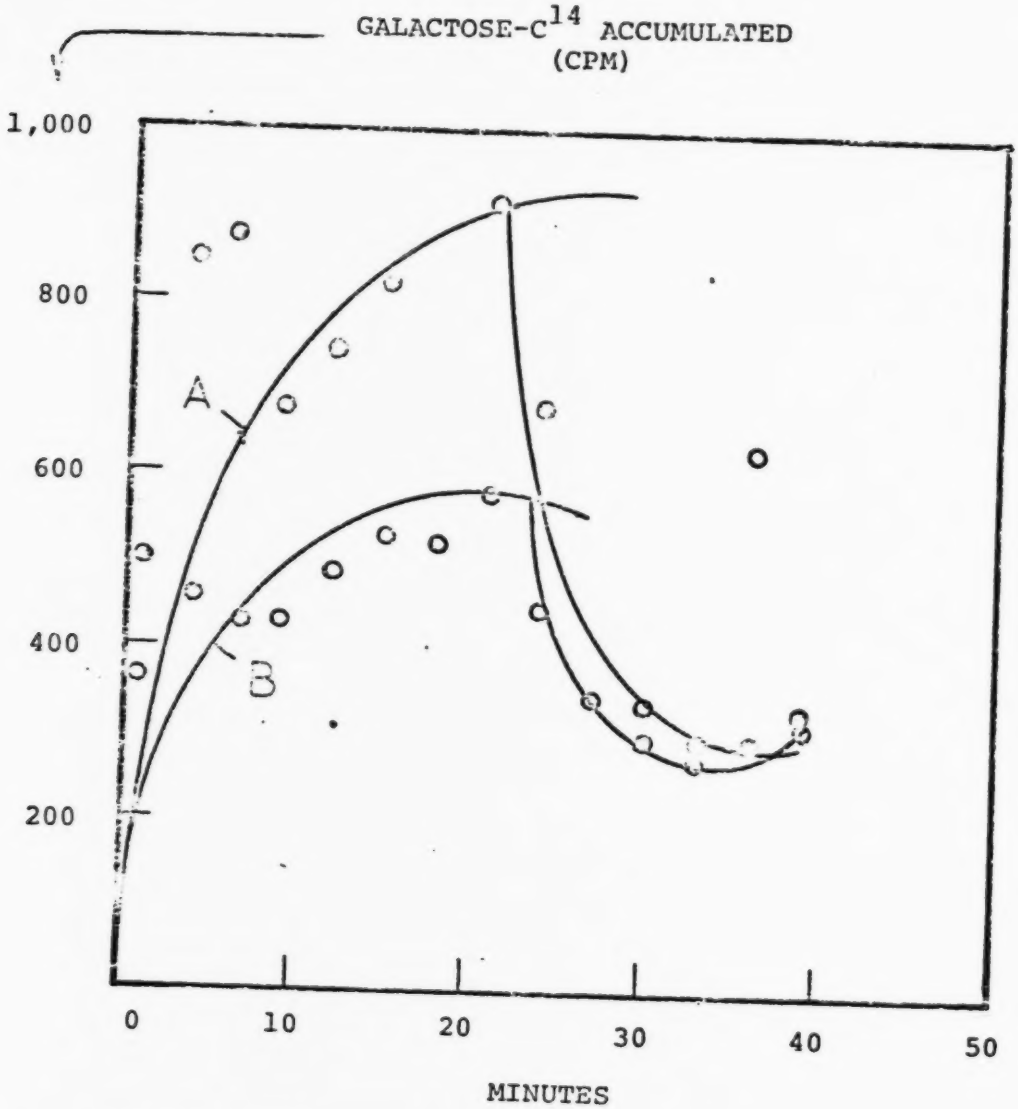


FIGURE 17. Comparison of galactose transport systems for cells grown in presence and absence of electric fields. At zero time a washed suspension of succinate-grown cells of E. coli ML 32,400 containing 0.306 MG of bacterial dry wt per ML was treated with a solution of C^{14}-galactose. After 23 minutes excess galactose-C-12 was added.

A. Test flask - AC current 120 MA
B. Control

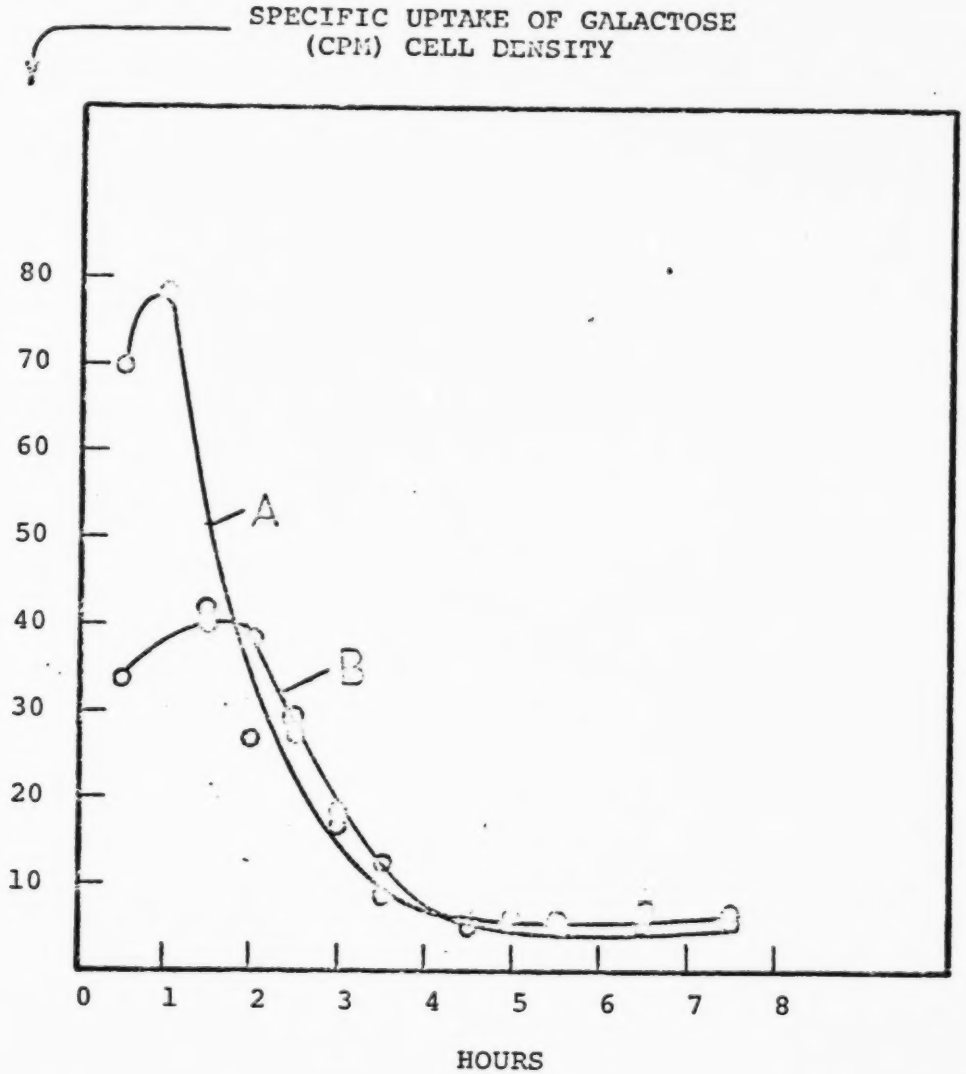


FIGURE 18. Development of galactose transport system during growth of E. coli ML 32,400. Uptake of galactose-C^{14} by cells (CPM/cell density) was measured during growth cycle.
Conditions: At zero time, 5 ml of inoculum, 0.1 ml of galactose C-12 ($4x10^{-3}$M) and 1.0 ml of galactose C-14 were added.

A. Test flask - AC current 120 ma.
B. Control flask.

SECTION VII

REFERENCES

Abood, L. G., R. W. Gerard, and S. Achs. "Electrical Stimulation of Homogenates and Particles," Am. J. Physiol., 171, 134 (1952).

Abood, L. G. "Mechanism of Inhibition of Phosphorylation in Brain Mitochondria by Electrical Impulses," Am. J. Physiol., 176, 247 (1954).

Abood, L. G., and L. Romanachek. "Inhibition of Oxidative Phosphorylation in Brain Mitochondria by Electric Currents and Effect of Chelating Agents and Other Substances," Biochem. J., 60, 233 (1955).

Carlson, S. "The Influence of Various Alternating Currents on the Enzymatic Activity of Yeast Under Aerobic Culture," Arch. Mikrobiol. 20, 221 (1954).

Carlson, S. "The Influence of Various Alternating Currents on the Oxidative Citric Acid Fermentation," Zbl. Bakt. II Abt. Orig., 569 (1960a).

Carlson, S. "The Influence of Low AC Current on Biosynthesis of Gluconic Acid by A. niger in Submerged Culture," Arch. F. Mikrobiol., 37, 7 (1960b).

Fritz, H. "On the Influence of Low and High Frequency Electrical Current on the Growth of Various Microorganisms," Z. Bakt. Parasitenk II Abt., 78, 386 (1929).

Gillissen, G., and S. Carlson. "Influence of An AC Field on Antibiotic Production by Molds in Surface Culture," Experientia, 8, 344 (1952a).

Gillissen, G., and S. Carlson. "Production of Antibiotics by Molds in Surface Culture under the Influence of Electric Currents," Comp. Rend., 234, 1994 (1952b).

Horecker, B. L., J. Thomas, and J. Monod. "Galactose Transport in E. coli," J. Biol. Chem., 235, 1580 (1960).

Hallum, J. A. "Treatment of Microorganisms," U.S. Patent 3,058,894 (1962).

V. Hattingberg, I. "Chrtersuchengen uber den Reizungs-stoffwechsel roter Blutzellen," Z. Biologil, 95, 44 (1934).

Kleiber, M. "Beitrag zur Trage der Einwirkung elektrischer Stiome Auf Mikro-Organism," Biochem. Z., 160, 312 (1925).

Krueger, A. P. "Some Biologic Properties of Gaseous Ions," 9. Albert Einstein Medican Center, 8, 79088 (1960).

Osterhout, W. J. V. "Water Absorption by Nereis limbata Eggs," J. Gen. Physiol, 33, 379 (1950).

Palladin, V. I., and G. I. Millyak. "Action of Electric Current on the Activity of the Enzymes of Alcoholic Fermentation," Bull. Acad. Sci., St. Petersburg, 8, 1592 p. 247 (1914).

Rosenberg, L. A., and I. B. Ulanovski. "Growth of Bacteria During Cathode Polarization of Steel in Water," Mikrobiologiya, 29, 721 (1960).

Schechter, V. "Electrical Control of Rhizoid Formation," J. Gen. Physiol.,18, 1 (1934).

Schieblich, M., and M. Schulze. "Betrage zur Einivirkung des Electrischer Stromes auf Bakterien," Biochem. Z., 168, 192 (1926).

Smith S., and E. Cook. "Magnetic Fields Increase Trypsin Activity," Chem. Eng. News., 44-45 (Jan. 21, 1963).

Spohr, G. "Electrical Stimulation of Bacteria," Water and Wastes Eng., 41 (April 1964).

Spohr, G. "Electrical Stimulation of Bacteria in Waste-water and Anaerobic Digestion," Water and Sewage Works (May 1965).

Thiele, H., and R. Wolf. "Effect of Electric Fields on Bacteria," Zbl. Bakt. I. Orig., 25, 650 (1899).

Wendel, K. "Increased Metabolism and Acid Activity in A. niger as a Function of Cultural Conditions," Zent. Bakt. II, 110, 612 (1957).